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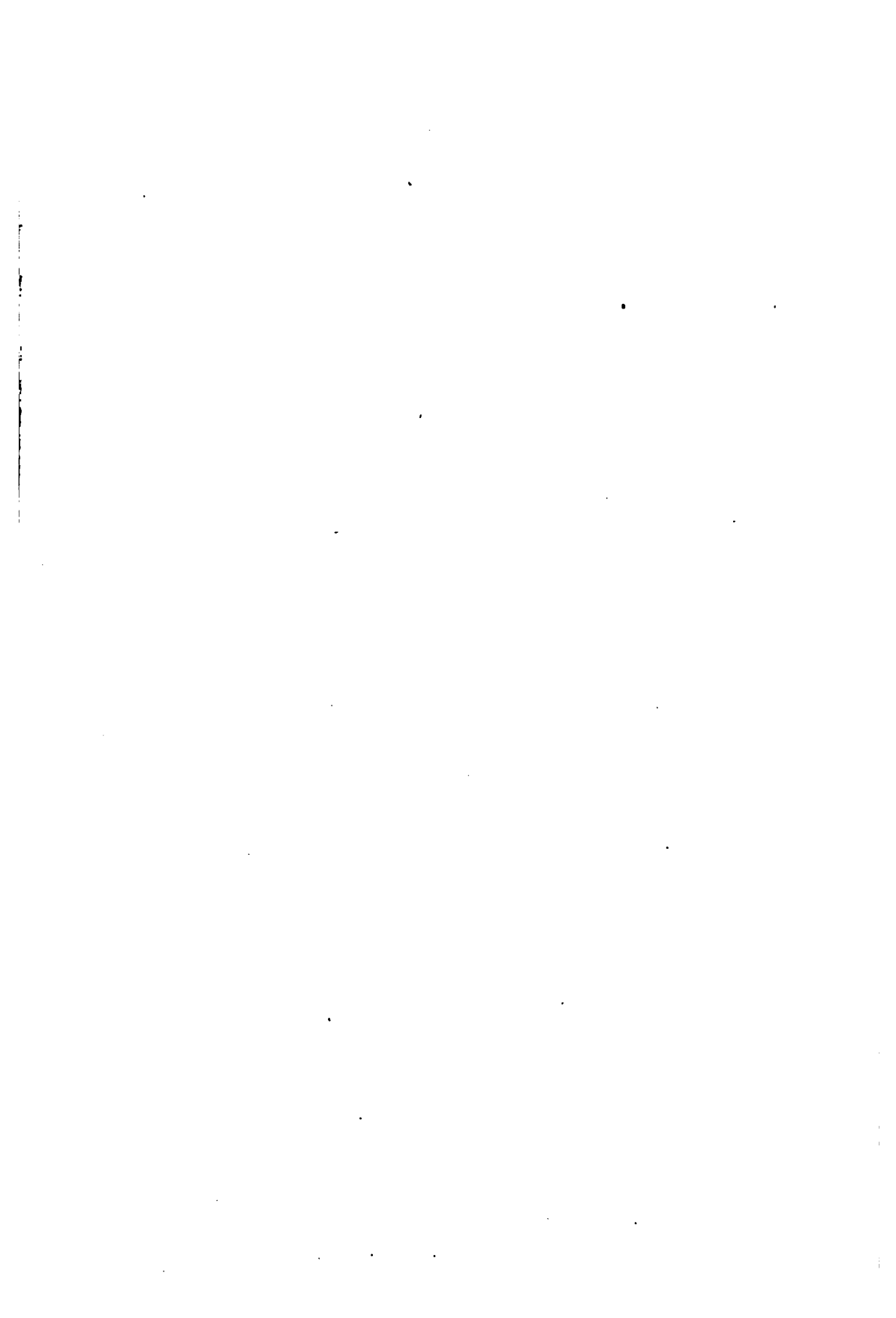
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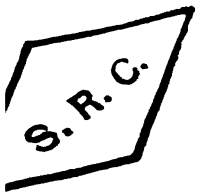
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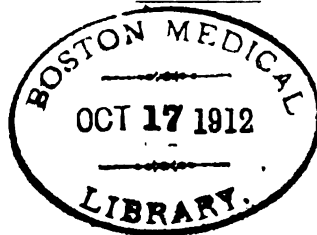


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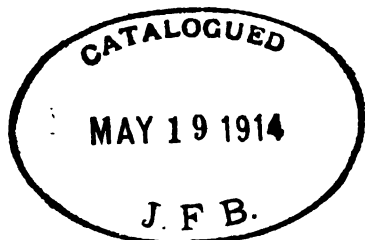
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# INDEX.

- Abnormal constituents of the urine, 1893, 1929, 2002.
- Accessories to the microscope, early, 1659.
- Acidity of roots, 2005.
- Admetus pumilio, development of, 2020.
- Agglutination of pneumococcus, 1866.
- Albugo, gametogenesis and fertilization in, 1704.
- Albumin in urine, test for, 1893.
- Algæ, unicellular, culture of, 1827.
- Allium cepa, outline for elementary study of, 1644.
- American Microscopical Society, review of trans. of 23rd ann. meet., 1674, 1714, 1771.
- Amitotic division, physiological investigation of, 1761.
- Amœba miurai in peritoneal tumors, 2061.
- Amœba, outline for elementary study of, 1852.
- Amœba, anesthesia of, 2088.
- Amphioxus, excretory organs of, 2061.
- Anærobic bacteria, methods of investigating, 1673.
- Anærobic bacteria, methods of cultivating, 1974.
- Anærobic bacteria, review of existing methods of cultivating, 1694, 1741, 1800, 1854.
- Anærobic bacteria, simple device for isolating, 1825.
- Analysis of urine, 1794, 1848, 1893, 1929, 2002.
- Anesthesia of animals, 2051, 2088.
- Anthoceros lævis, apospory in, 1663.
- Aplysia limacina, physiology of nervous system of, 1905.
- Apparatus:
- Cabinet for microscopical slides, 1726.
  - Centrifuges, hand and electric, 1849.
  - Centrifuge percentage tube, 2050.
  - Convenient case for butterflies and moths, 1615.
  - Convenient microscopical table, 1968.
  - Counter for bacteria colonies, 1970.
  - Demonstration eyepiece, 1648.
  - Dropper for use in cutting celloidin sections, 1917.
  - Exposure-test kit, improvised, 1685.
  - Fermentation tube for analysis of gas generated by bacteria, 1884.
  - For biology laboratory of small high school, 1603.
  - For collecting land and fresh water mollusca, 1954.
  - For cultivating anærobic bacteria, 1694, 1741, 1800, 1974.
  - For demonstrating respiratory ratio in starchy and oily seeds, 1927.
  - For elementary botany, 1639.
  - For elementary zoology, 1677.
  - For examination of sputum, 1981.
  - For filling pipettes in water analysis, 1919.
  - For isolating anærobic bacteria, 1825.
  - For making lantern slides, 1842.
  - For photographing gross anatomical specimens, 1791.
  - For photographing metals, 1925.
  - For photographing pathological and bacteriological specimens, 1683.
  - For preparation of bacteria, 1609, 1688.
  - For testing osmotic properties of colloidal solutions, 1942.
  - For testing sap pressure, 1988.
  - For washing, staining, and dehydrating small specimens, 1645.
  - For water analysis, 1982.
  - Hæmacytometer for counting blood corpuscles, 2049.
  - Hæmaglobinometer, Gower's, 2090.
  - Hæmometer, Fleisch's, 2090.
  - Incubator for individual use in large classes, 1914.
  - Incubator for maintenance of constant low temperature, 1972.
  - Laboratory desk, 1721.
  - Nessler cabinet, improved, 1918.
  - Photo-micrographic device, 1997.
  - Platinum strainer for use in Pal-Weigert method, 1986.
  - Pocket magnifier and pocket microscope, 1963.
  - Porte lumière, 1796.
  - Potometer, 2048.
  - Press for obtaining small amounts of plant juice, 1679.
  - Projection microscope, 1892, 1930.
  - Rack for exhibiting charts, 1993.
  - Simple device for storing fluid culture media, 1876.
  - Simple fixing oven for blood preparations, 1967.
  - Simple photo-micrographic camera, 1681.
  - Simple, vertical photo-micrographic camera, 1889.
  - Thermostat, easily constructed, 1965.
  - Trap for marine animals, 1862.
  - Ureometer, 1795.
  - Urinometer, 1794.
  - Vertical camera and its laboratory uses, 1782.
  - Watch-glass for embedding small objects, 2080.
  - X-Ray table, 1656.
- Araliaceæ, embryogeny in, 1938.
- Artificial sap pressure, 1988.
- Artificial imitations of protoplasmic activities and methods of demonstrating them, 1597.
- Asclepiadaceæ, development of pollen in, 1899.
- Asclepias, embryology of, 1858.

- Asclepias, pollen mother cells of, 1937.  
 Attachment to the Minot microtome for cutting sections of one micron thickness, 1994.  
 Autotomy and regeneration in the Phasmodæ, 1668.  
 Babes-Ernst bodies in bacteria, 2067.  
 Bacillus anthracis, technique for staining, 1731.  
 Bacillus coli communis, technique for staining, 1689.  
 Bacillus coli communis, morphology of, 1908.  
 Bacillus diphtheriæ, technique for staining, 1653.  
 Bacillus exiguum, 2036.  
 Bacillus halofaciens, 2033.  
 Bacillus tuberculosis, technique for staining, 1652.  
 Bacillus typhosis, technique for staining, 1687.  
 Bacillus typhosis, morphology of, 1908.  
 Bacillus of asiatic cholera, 1731.  
 Bacillus of influenza, technique for staining, 1793.  
 Bacteria, anærobic, review of existing methods of cultivating, 1694, 1741, 1800, 1854.  
 Bacteria, fixing, without heat, 2106.  
 Bacteria, influence of light in pigment production of, 1917.  
 Bacteria, internal structure of, 2068.  
 Bacteria of glanders, technique for staining, 1732.  
 Bacteria, outline for elementary study of, 1798.  
 Bacteria, scientific roll of, 1634.  
 Bacteria, structure of, 1672, 2067, 2068.  
 Bacteria, technique for preparation of, 1609.  
 Bacteria in the soil, 1975.  
 Bacterial colonies, method for fixing and sectioning, 1980.  
 Bacterial flora in freshly drawn milk, 2029, 2086.  
 Bacteriological laboratory of Cornell university, 1909.  
 Bacteriological Literature (reviews), 1632, 1672, 1712, 1825, 1865, 1918, 1945, 2026, 2067, 2105.  
 Beggiatoa alba, outline for elementary study of, 1700.  
 Beggiatoa mirabilis, cell structure in, 1704.  
 Biology laboratory in the small high school, 1603.  
 Biology laboratory of Morningside College, 1949.  
 Biology laboratory of Vassar College, 1717.  
 Blood, degeneration and regeneration of, 1705.  
 Blood, examination of, 2049, 2091.  
 Blood, investigation of origin and development of, 1939.  
 Blood, review of methods of staining, 2007, 2039, 2092.  
 Blood, staining, 2041.  
 Blood platelets, making preparations of, 1859, 1900, 1901.  
 Blood stain, Goldhorn's, 1635, 1716.  
 Bone, method of sectioning, 1781.  
 Botanical Literature (reviews), 1623, 1662, 1702, 1760, 1815, 1858, 1899, 1937, 2015, 2057, 2096.  
 Carbol fuchsin for staining bacillus tuberculosis, 1652.  
 Carbol fuchsin in general botanical work, 1727.  
 Carbon assimilation, 2084.  
 Cell, artificial, how to make, 2045.  
 Cell, Traube's, how to make, 2045.  
 Cell nucleus, oxidative properties of, 2063.  
 Cell studies: spindle for mationin Agave, 2016.  
 Chemical basis of variation, 2018.  
 Chemical fertilization or parthenogenesis in unfertilized eggs, 1630.  
 Chitin, development of, in insects, 2097.  
 Chloretone for anesthetising animals, 2051.  
 Chlorides, phosphates, and sulphates, testing urine for, 1848.  
 Chromic acid for fixing blood, 2009.  
 Chromosome reduction in Larix leptolepis, 1624.  
 Chronic interstitial pancreatitis, causes and varieties of, 2103, 2104.  
 Ciliated epithelium, action of fluorescent substances on, 1767.  
 Cirrhosis, non-suspension of glycogenic function of the liver in, 2066.  
 Cladophora, outline for elementary study of, 2094.  
 Cleaning slides, 1781.  
 Cleaning slides and cover-glasses, 1636.  
 Closterium, outline for elementary study of, 1896.  
 Collecting and preserving fungi, 2075.  
 Collodion sacs, improved method of making, 1713.  
 Convenient and economical cabinet for microscopical slides, 1726.  
 Convenient case for butterflies and moths, 1615.  
 Convenient method of washing, staining, and dehydrating small specimens, 1645.  
 Convenient microscope table, 1968.  
 Convenient press for obtaining small amounts of plant juice, 1679.  
 Corpuscles, method of counting, 2049.  
 Correlation of coloring in liver, skin, and hair, 2017.  
 Corroding action of roots, 2006.  
 Curare and physostigmin, mutual antagonism between, 1711.  
 Current Bacteriological Literature (reviews), 1632, 1672, 1712, 1825, 1865, 1908, 1945, 2026, 2067, 2105.  
 Current Botanical Literature (reviews), 1623, 1662, 1702, 1760, 1815, 1858, 1899, 1937, 2015, 2057, 2096.  
 Current Zoological Literature (reviews), 1627, 1666, 1707, 1764, 1819, 1861, 1902, 1940, 2019, 2061, 2099.  
 Cyanophyceæ, nuclear structure in, 1702.  
 Cyst originating from the ductus thysioglossus, 2024.  
 Cysticerci, structure of, 2062.  
 Cytodiagnosis, 1634.  
 Cytology, Embryology, and Microscopical Methods (reviews), 1625, 1664, 1705, 1762, 1817, 1859, 1938, 2017, 2059, 2097.  
 Degeneration of the Islands of Langerhans of the pancreas in diabetes, 1943.  
 Demonstration eye-piece, 1648.  
 Demonstration of nerve fibres in the ventral cord of the earthworm, 1614.  
 Dendrocornetes paradoxus, 1941.  
 Development of the egg and fertilization in Pinus strobus, 1623.

- Development of the pollen tube and division of generative nucleus in certain species of pines, 1623.
- Diatoms, outline for study of, 1853.
- Diffusion in plants, rate of, 2045.
- Directive influence of light on the earthworm, *Allolobophora foetida*, 1668.
- Double mounting of whole objects, 1926.
- Dragon flies of Illinois, 1766.
- Dropper for sterile fluids, 2019.
- Early development of the circulation in the suprarenal of the rabbit, 1625.
- Elastic tissue, normal and pathological processes in, 2103.
- Electric centrifuge for urinary analysis, 1849.
- Elementary Medical Micro-technique, 1608, 1652, 1686, 1730, 1793, 1848, 1893, 1929, 2002, 2049, 2090.
- Embedding small objects, 2080.
- Entomostraca of South America, fresh water, 1707.
- Erythronium, life history and cytology of, 1663.
- Eudorina elegans, outline for elementary study of, 2013.
- Euphorbia dulcis, embryology of, 2058.
- Evaporation, lifting power of, 2047.
- Evolution of plants, 1816.
- Examination of hairs for medico legal purposes, 2018.
- Examination of milk, 1793.
- Examination of water for typhoid bacilli, 1688.
- Experiments on synthesized media, 2026.
- Exposure-test kit, improvised, 1685.
- Exuvial glands and the formation of the exuvial fluids in insects, 2021.
- Fat metabolism, relation of lipase to, 1864.
- Fermentation tube for analysis of gas generated by bacteria, 1884.
- Fermentation tube in cultivation of anaerobic bacteria, 1805.
- Fertilization and development, 2015.
- Fertilization and development in Juglandaceae, 1858.
- Fertilizing enucleated fragments of eggs of *Cystosira barbata*, 1821.
- Fibrillae in plant protoplasm, 1815.
- Finish for laboratory table tops, 1651.
- Fixing blood by chemical fixatives, 2040.
- Fixing dry blood films, 2039.
- Fixing methods for blood, 2008, 2039.
- Fixing method for hydra, 1983.
- Flagella, method of staining, 1836.
- Flowering plants of Rocky mountains, analytical key to, 2058.
- Focusing the instrument in photo-micrography, 1728.
- Food formation, plant, effect of light on, 2084.
- Formulae :
- Carbol fuchsin, 1652, 1727.
- Culture medium for soil bacteria, 1975.
- Dahlia for staining blood, 2042.
- Ehrlich's eosin and hæmatoxylin mixture, 2093.
- Ehrlich's triacid stain, 2091.
- Eosin, aurentia, and nigrosin mixture, 2092.
- Fehling's solution, 1894.
- Fixative for zymogen granules, 1819.
- Gabbett's blue, 1652.
- Gentian violet, 1609, 1654.
- Goldhorn's polychrome methylen blue, 1635, 1716.
- Gram's decolorizing mixture, 1686.
- Hæmatoxylin and eosin stain, 2091.
- Hatai's fixative for spinal ganglion cells, 1828.
- Hermann's fluid for leucocytes and nuclei, 2041.
- Jenner's blood stain, 2091.
- Loeffler's alkaline methylen blue for staining blood, 1609, 2043.
- Mannaberg's hæmatoxylin for staining malaria blood, 2042.
- Mannaberg's picric acid and acetic acid fixative for blood, 2041.
- Methyl green for staining blood, 2043.
- Methyl green and fuchsin for staining blood, 2043.
- Methyl violet, 1609.
- Methyl violet and rubidin for staining blood, 2044.
- Methylen blue, 1638.
- Methylen blue for staining blood, 2043.
- Pitfield's flagella stain, 1836.
- Ripart and Petit's solution, 1641.
- Rumbler's methyl-green-eosin stain, 1628.
- Safranin for staining blood, 2044.
- Thionin for staining blood, 2044.
- Toison's fluid, 2049.
- Toluidin for staining blood, 2044.
- Ziehl's carbol fuchsin, 1727.
- Frambæ, pathology of, 2065.
- Gabbett's blue for staining tubercle bacilli, 1652.
- Gas production, easy methods of demonstration in test tubes, 1974.
- Gegarines, development of, 2061.
- Gelatin capsules for small specimens, 1817.
- Gelatin cover-glasses, 1763.
- General Physiology (reviews), 1629, 1668, 1710, 1767, 1821, 1863, 1904, 1942, 2022, 2063, 2101.
- Gentian violet, formula for, 1609, 1654.
- Germ cells, numerical law of, 2060.
- Germicidal action of organic peroxides, 1712.
- Ginkgo biloba, spermatozoid of, 1773.
- Goldhorn's polychrome methylen blue, 1635, 1716.
- Gonionemus murbachii, physiology of nervous system of, 2022.
- Gonococcus, technique for staining, 1690.
- Gram's method of staining pneumococcus, 1686.
- Growth of plants, methods of investigating, 1846, 1890.
- Hæmacytometer, Thoma-Zeiss, for counting red and white blood corpuscles, 2049.
- Hæmaglobinometer, Gower's, for estimating the hæmaglobin in blood, 2090.
- Hæmometer, Fleischl's, for estimating the hæmaglobin in blood, 2090.
- Hand centrifuge for urinary analysis, 1849.
- Hatai's fluid for fixing spinal ganglion cells, 1828.
- Helminths, action of, in intestines, 1902.
- Hemitrichia clavata, 1799.
- Heterogenesis and evolution, 1900.

- Hints on collecting land and fresh water mollusca, 1954.  
 Histology (reviews), 1631, 1670, 1769, 1905, 1943, 2024, 2065, 2103.  
 Holoblastic cleavages in ovum of *Cestracion*, 2018.  
 Hopkins Seaside Laboratory, 1869.  
 Human placentation, 1709.  
*Hydra viridis*, regeneration in, 1710.  
*Hydrodictyon reticulatum*, outline for elementary study of, 2093.  
 Illuminating the object in photomicrography, 1618.  
 Imbibition, effect of dissolved substances on, 2046.  
 Imbibition, effect of temperature upon, 2046.  
 Imbibition, rise of temperature during, 2046.  
 Implantation of the embryo in the uterus, 1709.  
 Improved method of making collodion tubes for dialyzing, 2038.  
 Improved method for staining flagella, 1836.  
 Incubator for maintenance of constant low temperature, 1972.  
 Incubator for use in water analysis, 1982.  
 Incubator, physician's, 1689.  
 Infectious bacteria, cultivating, 1865.  
 Influence of light in pigment production of bacteria, 1917.  
 Infusoria, anesthesia of, 2089.  
 Infusoria, ciliate, parasitic, in respiratory tree of *Holothuria californica*, 1765.  
 Injecting the blood vessels of the earthworm, 1736.  
 Injecting tubercle bacilli into trachea of dog, effect of, 1824.  
 Injection with cold liquid gelatin, 1625.  
 Ink for writing on glass, 1680.  
 Intensive coloration of bacillus of Koch, 1826.  
 Iodophilia, 1823.  
 Karyokinesis in *Magnolia* and *Liriodendron*, 1624.  
 Kitchen of the twentieth century, 1961.  
 Laboratories of pathology and bacteriology of the Medico-chirurgical College of Philadelphia, 2076.  
 Laboratory equipment for beginning course in zoölogy, 1677.  
 Laboratory equipment for elementary botany, 1640.  
 Laboratory equipment for the small high school, 1603.  
 Laboratory manual of botany, 2058.  
 Laboratory of plant physiology of the agricultural academy in Poppelsdorf-Bonn, 1829.  
 Laboratory outlines for the elementary study of plant structures and functions from the standpoint of evolution, 1639, 1698, 1798, 1852, 1896, 1934, 2013, 2055, 2093.  
 Laboratory Photography:  
   A form of vertical camera and its laboratory uses, 1782.  
   A photo-micrographic device, 1997.  
   A simple vertical photo-micrographic camera, 1889.  
   An apparatus for photographing gross anatomical specimens, 1791.  
   An improvised exposure-test kit, 1685.  
   An X-ray table, 1656.  
   New method of focusing in photomicrography, 2082.  
   Photographic apparatus for pathological and bacteriological specimens, 1683.  
   Photographing *Uredineæ* with the microscope, 1655.  
   Photo-micrography III, 1618; IV, 1728.  
   Photomicrography with simple apparatus, 1681.  
   Photo-microscopy of metals as practiced by steel companies, 1920.  
   Some suggestions on one way to make lantern slides, 1842.  
 Laboratory student's desk, 1721.  
 Laboratory tables, finishing the tops of, 1651.  
 Lead, micro-chemical analysis of, 1850, 1895, 1932, 2011, 2053.  
 Lepas, embryology of, 2100.  
*Leucapina crenulata*, anatomy of, 2100.  
 Leukemia, 1905.  
 Life by the seashore, an introduction to natural history, 1666.  
 Light, effect of, on carbon assimilation, 2085.  
 Light, effect of, on food formation, 2084.  
 Light, effect of, on starch formation, 2084.  
 Lights available for projection work, 1733.  
 Liliaceæ, fertilization in, 2057.  
 Lipogenesis, relation of lipase to fat metabolism, 1864.  
*Littorina*, negative phototaxis in, 1767.  
 Loeffler's alkaline methylen blue, 1609.  
*Loxosoma davenporti*, 1861.  
*Lycogala epidendrum*, 1799.  
*Lyngbya* sp., outline for elementary study of, 1699.  
 Microscopical mount for museum and class work, 1989.  
 Maddox, Richard Leach, gelatin-bromide dry plate of, 1947.  
 Malaria, investigation of, 1708, 1764, 1765.  
 Malaria blood, method of preparing specimens of, 1626.  
 Manual of determinative bacteriology, 1632.  
 Marble blocks for celloidin tissue, 1970.  
 Marine explorations of Prince Albert I<sup>er</sup> de Monaco, 1862.  
 Mast cells, method of staining, 1867.  
*Megastoma entericum*, methods of preparing, 1820.  
*Merismopedia* sp., outline for elementary study of, 1699.  
 Metals, photo-microscopy of, 1920.  
 Methods in Plant Physiology, 1846, 1890, 1927, 2004, 2045, 2084.  
 Methods:  
   Anesthetizing animals, 2051, 2088.  
   Cleaning slides, 1781.  
   Cleaning slides and cover-glasses, 1636.  
   Collecting and preserving fungi, 2075.  
   Collection, care, and preparation of rhizopods, 1940.  
   Collection, preparation and arrangement of mollusks, 1954.  
   Contrast staining with eosin and methylen blue, 1637.  
   Counting blood cells, 2049.  
   Cultivating anaerobic bacteria, 1694, 1741, 1800, 1854, 1974.  
   Cultivating infectious bacteria, 1865.

- Culture of unicellular algæ, 1827.
- Demonstrating dependence of plants upon oxygen for growth, 1846.
- Demonstrating evolution of heat by respiration, 1928.
- Demonstrating gas production in test-tubes, 1974.
- Demonstrating intra-molecular respiration, 1927.
- Demonstrating inter-cellular constituents of typhoid bacillus, 1865.
- Demonstrating intestinal glands in crustacea, 1707.
- Demonstrating protoplasmic activity artificially, 1597.
- Demonstrating respiration in chlorophyll-bearing plants, 1891.
- Demonstrating respiratory ratio in starchy and oily seeds, 1927.
- Demonstrating nerve fibers in ventral cord of earthworm, 1614.
- Detecting typhoid bacilli, 2106.
- Determining composition of gas evolved during respiration, 1891.
- Determining distribution of growth in roots 1890.
- Determining distribution of growth in stems and leaves, 1847.
- Determining effect of cold in cholera and typhoid bacilli, 1672.
- Determining elements necessary for nutrition of plants, 2004.
- Determining minimum, optimum and maximum temperature for growth of plants, 1847.
- Differentiating pneumococcus and streptococcus, 1712.
- Distinguishing methylen blue from methyl blue, 1725.
- Elementary study of plants, 1639, 1698, 1798, 1852, 1896, 1924, 2013, 2055, 2093.
- Embedding small objects, 2080.
- Embryology of *Lepas*, 2100.
- Embryology of the salmon, 2099.
- Embryology of trout, 2100.
- Estimating hæmaglobin in blood, 2090.
- Experiments on regeneration in *Hydra viridis*, 1710.
- Fertilizing enucleated eggs, 1821.
- Finishing laboratory table tops, 1651.
- Fixing and sectioning bacterial colonies, fungus mycelium, etc., 1980.
- Fixing and staining blood plates, 1901.
- Fixing and staining cell structures in Cyanophyceæ, 1702.
- Fixing and staining small specimens in gelatin capsules, 1817.
- Fixing and staining the parasite *Megastoma entericum*, 1820.
- Fixing bacteria without heat, 2106.
- Fixing blood by chemical fixatives, 2040.
- Fixing blood by dry heat, 2039.
- Fixing hydra, 1983.
- Fixing *Hydrophilus* eggs, 1665.
- Fixing spinal ganglion cells, 1828.
- Focusing in photomicrography, 1728, 2082.
- Formol methyl method in study of nerve fibers, 1632.
- Freeing a botanical laboratory from insect pests, 1828.
- Fungi, collecting and preserving, 2075.
- Hanging block for observing developing bacteria, 1713.
- Injecting the blood vessels of the earthworm, 1736.
- Injection with cold liquid gelatin, 1625.
- Investigating anærobic bacteria, 1673.
- Investigating the serum of pneumococcus, 1866.
- Investigating the typhoid bacillus, 1945.
- Intensive coloration of the bacillus of Koch, 1826.
- Isolating anærobic bacteria, 1825.
- Isolating typhoid bacillus, 2068.
- Making blood preparations, 2007.
- Making collodion sacs, 1713, 2038.
- Making lantern slides, 1842.
- Making permanent mounts of frozen sections, 1670.
- Making permanent preparations of blood, 1797.
- Making preparations of blood platelets, 1859.
- Making the first gelatin-bromide dry plate, 1947.
- Mounting microscopic objects for museum and class work, 1989.
- Mounting objects for examination from both surfaces, 1926.
- Mounting objects for temporary study, 2028.
- Mounting simple microscopical objects, 2108.
- Paraffin embedding in vacuum, 1762.
- Paraffin embedding with carbon bisulphide as penetrator, 1664.
- Preparation of internal organs of dried insects, 1647.
- Preparation of malaria blood, 1626.
- Preparation of sections of hydra for class work, 1613.
- Preparing bone sections, 1781, 1996.
- Preparing fish for dissection, 1676.
- Preparing gastric glands of fundus of mammals, 1762.
- Preparing microscopical material for microphotography, 2017.
- Preparing muscular tissue for microscopical study, 1763.
- Preparing plant material for museums and general demonstration purposes, 1885.
- Preparing radulæ of snails, 1708, 1766.
- Preparing vertebrate skeletons, 1836.
- Preserving natural colors in anatomical specimens, 1735.
- Preserving zymogen granules, 1819.
- Preventing curling of paraffin sections, 1948.
- Rearing marine larvæ, 1941.
- Recording cultures of bacteria genealogically for laboratory purposes, 1877.
- Sectioning fresh plant tissues, 2074.
- Sectioning without embedding, 2096.
- Staining axis cylinders of fresh spinal cord, 1987.
- Staining bacteria, 1652, 1686, 1730.
- Staining blood, 1939.

- Staining blood, review of methods, 2007, 2039, 2092.  
 Staining blood films and malaria parasites, 1769.  
 Staining blood preparations, 2041.  
 Staining blood with eosin and methylen blue, 1705.  
 Staining bone marrow, 1705.  
 Staining elastic tissue, 1817.  
 Staining fats, pomades, paraffin, etc., 1818.  
 Staining flagella, 1836.  
 Staining mast cells, 1867.  
 Staining tissue in bulk, 1705.  
 Staining with carbol fuchsin in botanical work, 1727.  
 Staining the chromatin of the malaria parasite, 1635.  
 Studying finer structures of cysticerci, 2062.  
 Studying living blood cells, 1900.  
 Studying malaria blood, 1764.  
 Studying spermatozoid of *Ginkgo biloba*, 1773.  
 Studying tardigrades, 2062.  
 Testing osmotic properties of colloidal solutions, 1942.  
 Testing sap pressure, 1988.  
 Testing urine for albumin, 1893.  
 Testing urine for sediments, 1929.  
 Testing urine for sugar, 1894.  
 Testing warmth sensitivity of different parts of the human body, 1904.  
 To show dispensability of gravity in development of toad's egg, 2059.  
 Washing, staining, and dehydrating small specimens, 1645.  
 Methyl violet, formula for, 1609.  
 Methylen blue as an intra vitam stain for bacilli, 1766.  
 Micro-Chemical Analysis, 1610, 1649, 1738, 1850, 1895, 2011, 2053.  
 Micrococcus varians lactis, 2033.  
 Microscope and its revelations, 1628.  
 Microscopes, early, 1621.  
 Microscopical exhibit at the New York Botanical Garden, 1990.  
 Microscopical table, 1968.  
 Migrations of birds, precise direction taken in, 1768.  
 Milk, bacterial flora in freshly drawn, 2029, 2086.  
 Milk, bacteriological study of, 1793.  
 Modern bacteriological laboratory, 1909.  
 Modification of eosin and methylen blue contrast staining with technique, 1637.  
 Mollusca, collecting, 1954.  
 Monochlorial twins, 2059.  
 Monotropia uniflora, fertilization in, 1937.  
 Mosquito, adult female, anatomy and histology of, 1765.  
 Mounting objects for temporary study, 2028.  
 Mounting simple microscopical objects, 2108.  
 Movements of the intestine studied by means of the Röntgen Rays, 2023.  
 Museum, 2069.  
 Multiple myeloma, 1824, 1907.  
 Myelocytes in acute infectious diseases, 2025.  
 Myxinoidea, morphology of, 1861.  
 Necturus, development of skull of, 1861.  
 Nerve fibers, formol-methyl method in study of, 1632.  
 Nessler cabinet, improved form, 1918.  
 Neutral red in the examination of water, 1945.  
 New colony counter, 1970.  
 New ether freezing apparatus for the microtome, 2018.  
 New laboratory of plant physiology of the agricultural academy in Poppelsdorf-Bonn, 1829.  
 New method of embedding small objects, 2080.  
 New pathological and bacteriological laboratories of the Medico-chirurgical College, Philadelphia, 2076.  
 Nitrogen assimilation, 2026.  
 Nitrogen fixing bacteria, 1966.  
 Normal and Pathological Histology (reviews), 1631, 1670, 1769, 1905, 1943, 2024, 2065, 2103.  
 Note on photographing fruits, 1686.  
 Notes on technique, 1613, 1736.  
 Notes on the microscope, 1621, 1659.  
 Nutrient medium for cultivating anaerobic bacteria, 1812.  
 Nutrition of plants, method of determining, 2004.  
 Oculars for general laboratory work, 1646.  
 Orthonectides, studies on, 1819.  
 Oscillatoria sp., outline for elementary study of, 1700.  
 Osmometer for plant physiology, 2045.  
 Osmosis, physiological, 2046.  
 Osmotic properties of colloidal solutions, 1942.  
 Osmotic strength of cell sap in terms of potassium nitrate, 2046.  
 Otocyst of decapod crustacea, 1666.  
 Outlines of botany for the high school laboratory and class-room, 2016.  
 Pancreas in diabetes, 1943, 1944.  
 Pandorina morum, outline for elementary study of, 1935.  
 Paraffin embedding in vacuum, 1762.  
 Paraffin embedding with carbon bisulphide as penetrator, 1664.  
 Parthenogenesis in flowering plants, 2096.  
 Pathology of bubonic plague, 1631.  
 Pedicularium boryanum, outline for elementary study of, 1643.  
 Petromyzon fluviatilis, preparing eggs of, 1706.  
 Philotria canadensis, outline for elementary study of, 1643.  
 Phosphates, chlorides and sulphates, testing urine for, 1848.  
 Photographic apparatus for pathological and bacteriological specimens, 1683.  
 Photographing fruits, 1686.  
 Photographing gross anatomical specimens, 1791.  
 Photographing objects under liquids, 1782.  
 Photographing uredineæ with the microscope, 1655.  
 Photography in the deep sea, 1666.  
 Photomicrographic device, 1997.  
 Photomicrography, III, 1618; IV, 1728.  
 Photomicrography with simple apparatus, 1681.  
 Photomicroscopy of metals as practiced by steel companies, 1920.  
 Physical osmosis and imbibition, 2045.

- Physiological action of radiations of short wave lengths, 1630.  
 Physiological effects of valency and electrical changes of ions, 1822.  
 Physiological osmosis, 2046.  
 Physiology (reviews), 1629, 1668, 1710, 1767, 1821, 1863, 1904, 1942, 2022, 2063, 2101.  
 Physiology by the laboratory method for secondary schools, 2101.  
 Plankton of the north Atlantic ocean, 1627.  
 Plant juice, press for obtaining small quantities of, 1679.  
 Plant physiology, methods in, 1846, 1890, 1927, 2004, 2045.  
 Plant physiology, teaching, to large elementary classes, 2028.  
 Plant preparations for museums and general demonstration work, 1885.  
 Plasmodium, 1799.  
 Plasmolysis, 2046.  
 Plating as a means of determining the number of bacteria in drinking water, 1825.  
 Platinum strainer for use with sections which are to be prepared in accordance with the Pal-Weigert method, 1086.  
 Pleurococcus vulgaris, outline for elementary study of, 1698.  
 Pneumococcus, technique for examining, 1654, 1686.  
 Pneumococcus and streptococcus, differentiation of, 1712.  
 Pocket magnifier and pocket microscope, 1963.  
 Polychrome methylen blue, Goldhorn's, 1635, 1716.  
 Porte lumière for projection work, 1796.  
 Potometer for measuring rate of transpiration, 2048.  
 Practicum, handbook of practical botany, 1815.  
 Primary sarcoma of the tail of the pancreas, 1944.  
 Preparation of bone sections, 1781, 1906.  
 Preparation of internal organs of dried insects, 1647.  
 Preparation of sections of hydra for class work, 1613.  
 Preparing fish for dissection, 1676.  
 Preparing vertebrate skeletons, 1836.  
 Professional spirit and publication, 2107.  
 Projection microscopes using electric arc or oxyhydrogen light, 1892, 1930, 2012.  
 Prolongation of life of unfertilized egg of sea urchin by potassium cyanide, 1822.  
 Proteid molecule, chemistry of, 2023.  
 Proteolytic enzymes in animal tissues, 1711.  
 Protoplasmic activities, artificial imitations of, 1597.  
 Protoplasmic connections in plants, 1937.  
 Rack for exhibiting charts, 1993.  
 Radula of mollusks, embryonal development, 1766.  
 Reaction to stimuli in unicellular organisms, 1669.  
 Regeneration in the Phasmidæ, 1668.  
 Relation of physical chemistry to physiology and pathology, 1669.  
 Respiration in plants, methods for investigating, 1891, 1927.  
 Review of existing methods of cultivating anaerobic bacteria, 1694, 1741, 1800, 1854.  
 Review of methods of staining blood, 2007, 2039, 2092.  
 Revue Generale du Lait, 1633.  
 Rhizopod fauna of Leman basin, 1940.  
 Romanowsky staining of blood films and malarial parasites, 1769.  
 Root hairs, 2045.  
 Rubiaceæ, comparative embryology of, 1899.  
 Salmo fario, yolk organ of, 2098.  
 Salmon, embryology of, 2099.  
 Sarcoma of the thyroid, transplantation of, 1906.  
 Sarcomata of the skin, 2025.  
 Scenadesmus quadricauda, outline for elementary study of, 1853.  
 Sea beach at ebb tide, sea weeds and animal forms found on, 1666.  
 Sectioning fresh plant tissues, 2074.  
 Sectioning without embedding, 2096.  
 Sediments in the urine, 1929.  
 Sensitivity of different regions of the human body to warmth, 1904. •  
 Silver, micro-chemical analysis of, 1610, 1649, 1738.  
 Simple device for storing fluid culture media, 1876.  
 Simple fixing oven for blood preparations, 1967.  
 Simple form of dropper for use in cutting celloidin sections, 1917.  
 Simple methods of preparing bone sections, 1996.  
 Slime moulds, outline for elementary study of, 1799.  
 Solar projection apparatus and its adjustment, 1795, 1844.  
 Some improvements upon apparatus for water analysis, 1918.  
 Spermatogenesis and fertilization in *Zamia*, 1760.  
 Spermatozoa, enzyme of, causing development of mature eggs, 1821.  
 Spermatozoid of Ginkgo, 1773.  
 Spermatozooids of plants, 1662.  
 Sphærella pluvialis, outline for elementary study of, 1934.  
 Spirogyra, outline for elementary study of, 1897.  
 Sputum investigations, apparatus for, 1981.  
 Staining axis cylinders of fresh spinal cord, 1987.  
 Staining blood, methods of, 2007, 2041.  
 Staining blood films and malaria parasites, 1769.  
 Starch formation, effect of light on, 2084, 2085.  
 Stemonitis fusca, 1799.  
 Sterilizer, physician's hot air, 1688.  
 Sterilizer, physician's steam, 1689.  
 Sugar in urine, testing for, 1894.  
 Sulphates, phosphates and chlorides, testing urine for, 1848.  
 Sympathetic nervous system, physiology and morphology of, 1629.  
 Syncoryne sarsii, structure of, 1862.  
 Synopses of North American invertebrates, 1628.  
 System of recording cultures of bacteria genealogically for laboratory purposes, 1877.  
 Tardigrades, preparation of, for study, 2062.

- Technique of biological projection and anesthesia of animals, 1690, 1733, 1795, 1844, 1892, 1930, 2012, 2051, 2088.  
Temperature, effects of low, on Stentor and Asterias, 1768.  
Temperature, effect of, upon imbibition, 2046.  
Test tube basket, 1689.  
Tetanus, technique for staining, 1730.  
Thermostat, an easily constructed, 1965.  
Tissue-tension, 2047.  
Toad's egg, development of, 2059.  
Toxins and anti-toxins, 2102.  
Tradescantia, outline for elementary study of, 1645.  
Transactions of American Microscopical Society, Vol. XXII, 1674, 1714, 1771.  
Transpiration, 2047, 2048.  
Traube's cell, 2045.  
Trichinosis, 2066.  
Trichinosis, frequency in the United States, 1671.  
Trout, embryology of, 2100.  
Trypanosoma bruchii, the organism found in Nagana, or the Tse-tse fly disease, 2019.  
Tube casts in the urine, 2003.  
Tuberculosis, innoculability of, 2027.  
Typhoid bacillus, detecting, 2106.  
Typhoid bacillus, differential diagnosis of, 2068.  
Typhoid bacillus, intracellular constituents of, 1865.  
Typhoid bacillus, isolation of, 2068.  
Typhoid bacillus, method of investigating, 1945.  
Typhoid bacillus, morphology of, 1908.  
Unicellular organisms, reactions to stimuli in, 2102.  
Uredineæ, collecting and preserving, 2075.  
Ureometer, 1795.  
Uric acid, testing urine for, 1848.  
Urinary analysis, 1794, 1848, 1893.  
Urinometer, 1794.  
Variegated leaves, relation of, to carbon assimilation, 2085.  
Vassar College, biology laboratory of, 1717.  
Vaucheria sessilis, outline for elementary study of, 2055.  
Vegetable and animal cells, 1984.  
Volvox globator, outline for elementary study of, 2014.  
X-Ray table, 1656.  
Yellow fever, etiology of, 1908.  
Yolk organ of Salmo fario, 2098.  
Zamia, spermatogenesis and fertilization in, 1760.  
Zeitschrift fuer allgemeine physiologie, 1863.  
Zoölogical Literature (reviews), 1627, 1666, 1707, 1764, 1819, 1861, 1902, 1940, 2019, 2061, 2099.  
Zoölogy, laboratory equipment for beginning course in, 1677.  
Zymogen granules, preserving, 1819.

## INDEX OF AUTHORS.

- ALLEGER, W. W.  
Influence of Light in Pigment Production of Bacteria, 1917.
- BARBOUR, ERWIN HINCKLEY.  
A Pocket Magnifier and a Pocket Microscope, 1968.
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Some Apparatus Used in the Laboratory of the Buffalo Department of Health, 1981.
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Artificial Sap Pressure, 1888.  
A Photo-Micrographic Device, 1997.  
Some Observations on the Seventeen Year Locust, 2000.
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A Convenient Microscope Table, 1968.
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The Biology Laboratory in the Small High School, 1603.
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Vegetable and Animal Cells, 1984.
- BURKHOLDER, J. F.  
Preparation of Bone Sections, 1781.
- BURR, ROLLIN H.  
Modification of Eosin and Methylen Blue Contrast-staining, with Technique, 1637.
- BUXTON, B. H.  
Bacteria in the Soil, 1975.  
A Photographic Apparatus for Pathological and Bacteriological Specimens, 1683.
- CALVERT, PHILIP P.  
A Hint for the Preparation of Internal Organs of Dried Insects, 1647.
- CAMPBELL, JOHN P.  
Carbol Fuchsin in General Botanical Work, 1727.
- CHAMBERLAIN, CHARLES J.  
Current Botanical Literature (reviews), 1623, 1662, 1702, 1760, 1815, 1858, 1896, 1937, 2015, 2057, 2096.
- CHAMOT, E. M.  
Micro-Chemical Analysis:  
XIX. The Common Metals—Silver Group, 1610, 1649, 1738.  
XX. Silver Group (continued)—Lead, 1850, 1895, 1932, 2011, 2053.
- CLAYPOLE, AGNES M.  
Cytology, Embryology, and Microscopical Methods (reviews), 1625, 1664, 1705, 1762, 1817, 1859, 1899, 1938, 2017, 2059, 2097.
- COLE, A. H.  
The Technique of Biological Projection and Anesthesia of Animals, 1690, 1733, 1794, 1844, 1892, 1930, 2012, 2051, 2088.
- CONN, H. W.  
Current Bacteriological Literature (reviews), 1632, 1672, 1712, 1825, 1865, 1908, 1945, 2026, 2067, 2105.
- COX, ULYSSES O.  
A Convenient and Economical Cabinet for Microscopical Slides, 1726.
- CUMMING, M.  
Bacterial Flora of Freshly Drawn Milk, 2029, 2086.
- DAVISON, ALVIN.  
A Macroscopical Mount for Museum and Class Work, 1889.
- DENNIS, D. W.  
Photo-Micrography:  
III. Illuminating the Object, 1618.  
IV. Focusing the Instrument, 1728.  
Some Suggestions on One Way to Make Lantern Slides, 1842.
- ELLIOTT, L. B.  
A Simple Vertical Photo-Micrographic Camera, 1889.  
Notes on the Microscope:  
I. Early Microscopes, 1621.  
II. Early Accessories, 1659.
- FITZ-RANDOLPH, R. B., and WILSON, E. H.  
Incubator for the Maintenance of Constant Low Temperatures, 1972.
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Further Notes on a New Method of Focussing in Photomicrography, 2082.
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A Rack for Exhibiting Charts, 1993.
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A Simple Fixing Oven for Blood Preparations, 1967.
- GOLDEN, KATHERINE E.  
Photomicrography with Simple Apparatus, 1681.
- GRATACAP, L. P.  
The Museum, 2069.
- HARRISON, F. C., and CUMMING, M.  
An Easy Method of Demonstrating Gas Production in Test-Tubes, 1974.  
Bacterial Flora of Freshly Drawn Milk, 2029.  
Note on a Method of Cultivating Anaerobic Bacteria, 1974.
- HAY, W. P.  
An Easily Constructed Thermostat, 1965.
- HEALD, F. D.  
A Convenient Press for Obtaining Small Amounts of Plant Juice, 1679.
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Collecting and Preserving Fungi, 2075.  
Photographing Uredinæ with the Microscope, 1655.

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A Modern Bacteriological Laboratory, 1909.
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An Improvised Exposure Kit, 1685.  
Note on Photographing Fruits, 1686.
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The Microscopical Exhibit at the New York Botanical Garden, 1990.
- HUBBERT, WILLIAM R.  
Ink for Writing on Glass, 1680.
- HUNZIKER, OTTO F.  
A Review of the Existing Methods of Cultivating Anaerobic Bacteria, 1694, 1741, 1799, 1854.
- JENNINGS, H. S.  
Artificial Imitations of Protoplasmic Activities, and Methods of Demonstrating Them, 1597.
- JOHNSON, A. B.  
An X-Ray Table, 1656.
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A Fixation Method for Hydra, 1983.
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A Method for Cleaning Slides, 1781.
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An Improved Method of Making Collodion Tubes for Dialyzing, 2038.  
A Method for Fixing and Sectioning Bacterial Colonies, Fungus Mycelium, etc., 1980.
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An Improved Method for Staining Flagella, 1836.
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Elementary Medical Micro-Technique, 1608, 1652, 1686, 1730, 1792, 1848, 1893, 1929, 2002, 2049, 2090.
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Current Zoological Literature, (reviews), 1627, 1666, 1707, 1764, 1819, 1861, 1902, 1940, 2019, 2061, 2099.
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A new method of Embedding Small Objects, 2080.
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Laboratory Equipment for Beginning Course in Zoology, 1677.
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The Hopkins Seaside Laboratory, 1869.
- McFARLAND, JOSEPH.  
New Pathological and Bacteriological Laboratories of the Medico-Chirurgical College, Philadelphia, 2076.
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The Spermatozoid of Ginkgo, 1773.
- MURBACH, L.  
A Demonstration Eyepiece, 1648.
- OSBORN, H. L., and TYRRELL, C. C.  
On Preparing Vertebrate Skeletons, 1836.  
Staining Axis Cylinders of Fresh Spinal Cord, 1987.
- PEARL, RAYMOND.  
General Physiology, (reviews), 1629, 1668, 1710, 1767, 1821, 1863, 1904, 1942, 2022, 2063, 2101.
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Notes on Technique, 1613, 1736.
- PERKINS, H. F.  
Double Mounting for Whole Objects, 1926.
- PIERCE, NEWTON B.  
Sectioning Fresh Plant Tissues, 2074.
- PRATT, JOSEPH H.  
Normal and Pathological Histology, (reviews), 1631, 1670, 1769, 1823, 1905, 1945, 2024, 2065, 2103.
- REED, HOWARD S.  
Methods in Plant Physiology, 1846, 1890, 1927, 2004, 2045, 2084.  
Plant Preparations for Museums and General Demonstration Work, 1885.
- REESE, A. M.  
A Simple Form of Dropper for Use in Cutting Celloidin Sections, 1917.
- REIGHARD, JACOB.  
A Form of Vertical Camera and its Laboratory Uses, 1782.
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Photo-Microscopy of Metals as Practiced by Steel Companies, 1920.
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A Simple Device for Storing Fluid Culture Media, 1876.  
Fermentation Tube for Analysis of Gases Generated by Bacteria, 1884.
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The Kitchen of the Twentieth Century, 1961.
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A New Colony Counter, 1970.
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Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution, 1639, 1698, 1797, 1852, 1934, 2013, 2055, 2093.  
Oculars for General Laboratory Work, 1646.
- SLONAKER, J. ROLLIN.  
A Convenient Case for Butterflies and Moths, 1615.  
A Convenient Method for Washing, Staining, and Dehydrating Small Specimens, 1645.  
An Attachment to the Minot Microtome for Cutting Sections of One Micron Thickness, 1994.

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Some Improvements upon Apparatus for  
Water Analysis, 1918.
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Marble Blocks for Celloidin Tissues, 1970.
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Focussing in Photomicrography, 2082.
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The Biology Laboratory of Vassar Col-  
lege, 1717.
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On Preparing Vertebrate Skeletons, 1836.
- WALKER, BRYANT.  
Hints on Collecting Land and Fresh  
Water Mollusca, 1954.
- WALKER, ERNEST L.  
A Review of the Methods of Staining  
Blood, 2007, 2039, 2092.
- WARD, FREDERICK S.  
An Apparatus for Photographing Gross  
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# Applied Microscopy and Laboratory Methods

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## Artificial Imitations of Protoplasmic Activities, and Methods of Demonstrating Them.

Activities of the same character as many of those shown by some of the lower organisms, such as *Amœba*, may be produced through the action of chemical and physical laws. Experiments demonstrating this fact are valuable for an analysis of the behavior of these creatures, and many of those worked out by recent investigators (notably by Rhumbler) are very easily performed. A practical knowledge of them deserves to be more widespread than seems at present to be the case. The following are some of the experiments which the author is accustomed to demonstrate to classes in biology and general physiology:

1. *Amœboid Movements.* *Amœba* may be regarded, from a material standpoint, as a chemically active mass of viscid fluid which is continually interchanging substance with the medium surrounding it. It is constantly taking oxygen and other substances from the water, constantly giving off carbon dioxide and other excreted substances. Could such a mass be reasonably expected to change its form and move about? We may answer this question experimentally by providing an inorganic drop which fulfills these conditions, and observing it. The simplest and most satisfactory method which the author has found for doing this is to employ a drop of clove oil mounted on an ordinary glass slide in a mixture of three parts glycerin with one part 96 per cent. alcohol, and covered with a cover-glass. The clove oil and alcohol are miscible, so that a little alcohol is continually passing into the drop of clove oil, a little of the clove oil out into the alcohol; the conditions proposed above are therefore satisfied. The glycerin acts simply as a neutral medium to prevent too rapid interaction of the clove oil and alcohol, and by its thicker consistency to prevent the movements from taking place too rapidly.

Such a drop of clove oil will change its form, send out "pseudopodia," and creep about much as *Amœba* does. At first it may be circular, then a long projection will be sent out on one side, the entire drop may elongate, and progress as a whole in that direction. Currents may be formed within it, "pseudopodia"

may extend in several directions at once ; at times the drop may divide,—as also happens in *Amœba* ! Altogether, the drop of clove oil imitates with some degree of closeness the behavior of *Amœba*. The movements are of course due to local alterations in the surface tension of the drop of clove oil, owing to its irregular mixing with the alcohol.

As to the details of the experiment, the following may be said :

Pure clove oil is used. For the glycerine-alcohol mixture, use either three parts glycerine to one part 96 per cent. alcohol, or two parts glycerine to one part 70 per cent. alcohol. If the movements are later found to be too rapid and violent, add some water, or more glycerine. If the movements are too slow, add a little more alcohol to the mixture.

Proceed as follows : Place upon a slide two fine glass rods, for supporting the cover-glass. These may very conveniently be fastened to the slide, at a proper distance apart, with balsam. Place some of the glycerine-alcohol mixture on the slide, and cover with a cover-glass. Draw an ordinary medicine dropper out to a fine capillary point. Take up some of the clove oil with this, insert the point beneath the cover-glass, and inject a drop of the clove oil into the glycerine and alcohol. It will usually begin at once to change form and move about.

The thickness of the glass rods supporting the cover-glass is of importance ; if these are too thick, the movements will not be marked. Experiments should be made with rods of various thicknesses.

I have found this method of imitating protoplasmic movements much simpler than the preparation of Bütschli's emulsion of potassium carbonate and old olive oil, which is often difficult to prepare unless olive oil of exactly the right degree of rancidity is at hand.

*2. Control of the Direction of Movement.* With the drop of clove oil prepared as above, various methods can be illustrated by which the direction of movement might be controlled, in a mass which moves through changes in its surface tension. That a local chemical change taking place within the *Amœba* (producing thus a new chemical substance in a certain area) might cause the formation of a pseudopodium, and movement in a certain direction, may be illustrated by introducing some chemically different substance into a certain region of the drop of clove oil. A very satisfactory method is to inject a little 70 per cent. alcohol into the drop near one side. Take up a small drop of the alcohol in a pipette drawn to a *very* fine point ; introduce this beneath the cover-glass and into the drop, and press out a very little of the alcohol into the drop, removing the pipette at once. If this is skilfully done, and not too much alcohol is added, the drop will at once send out a "pseudopodium" on the side nearest which the alcohol was introduced, and often follows this up by moving in that direction. Of course if the alcohol (or any substance having less surface tension than the clove oil) could have been produced through a chemical change within the clove oil, the resulting movement would be the same.

Some practice is necessary to perform this experiment successfully. It is particularly important to use a freshly mounted drop of clove oil, which still shows some "spontaneous" movement. After a time the drop seems to become covered with a sort of incrustation which prevents its free movement. It is also

of great importance not to add too much alcohol ; if this is done, the drop will "explode" and dissolve in place of sending out a projection.

The movements of *Amœba* are of course largely controlled by external conditions. That this power of responding to external changes by directed movement is by no means peculiar to protoplasm or to living things, may be well illustrated with the drop of clove oil. *Amœba* regulates its movements according to the chemical substances present in the surrounding medium, moving towards some, away from others. The same is true for the drop of clove oil. If a drop of weak alcohol is introduced with the capillary pipette near to the clove oil, the latter will at once send out a "pseudopodium" in that direction, and will usually follow this up by moving as a whole toward the alcohol. *Amœba* regulates its movements with reference to the comparative temperature of different parts of the region where it is found. This also is true for the drop of clove oil. If a small portion of the slide is heated, as by holding the end of a hot wire against the cover-glass near to the drop of oil, the latter will send out a "pseudopodium" and begin to travel in the direction of the heated spot.

In injecting the alcohol, it is important not to inject too much, and to bring it very near to the drop of oil. For the movements toward a heated region, make a loop or ball at the end of a small wire ; heat this hot in the flame of an alcohol lamp, then touch the cover-glass not far from the drop of clove oil. The wire must be still very hot when it touches the cover. For all these experiments, use freshly mounted active drops.

3. *Choice of Food.* One of the most striking phenomena in the behavior of *Amœba* is its power of selecting substances which shall serve as food. *Amœba* takes its food simply by sending out pseudopodia, flowing around, and enveloping small bodies. But it by no means takes these at random ; sand, decayed plant tissue, bits of wood, dirt, etc., are as a rule rejected, while small living plant and animal cells, diatoms, infusoria, are enveloped, carried away, and digested. It thus shows a distinct choice in the substances which it takes into itself, and the power of choice has often been considered evidence of a rather highly developed mind.

Before accepting this conclusion for *Amœba*, it will be wise to test this matter of the power of choice for other fluids. A drop of chloroform is a good subject for experimentation. With a medicine dropper a drop of chloroform may be placed in the bottom of a watch-glass of water, and then with fine tweezers we may offer it various substances to test its power of choice. The whole proceeding may seem at first thought very absurd, but the results are striking.

We may first offer the drop of chloroform a fragment of glass ; this is held with the tweezers against the surface of the drop. It is not accepted. We push the glass against the drop, but the latter withdraws its surface from it so far as possible. We force the bit of glass into the drop of chloroform and let go of it. It is at once thrown out with energy. We try a small piece of wood in the same way ; it is rejected as decidedly as was the glass. We may now try a hard piece of gum shellac. This is accepted,—eagerly, one had almost said. Hardly has an angle of the piece of shellac touched the surface of the drop, when the latter literally reaches out, envelops the shellac, and draws it into itself. If we take

hold of the piece of shellac again with the forceps and draw it away, the chloroform drop stretches out after it, and lets go of it only with the greatest apparent reluctance. If allowed to retain the bit of shellac, it proceeds slowly to dissolve it,—just as the *Amœba* proceeds to digest the substance which it has taken within itself. A second and third piece of shellac will be accepted with the same avidity as the first.

Other substances may be offered to the chloroform drop. Glass, sand, dirt, wood, grass, gum arabic, and chlorate of potash, for examples, are rejected; shellac, paraffin, styrax, hard Canada balsam, and various other substances are accepted.

It thus appears that a drop of chloroform exercises choice in determining what substances shall be taken into itself, fully as decidedly as *Amœba* does. The same is true of other fluids, of whatever sort. We must then throw out completely the power of choice of food as any test of mental power or even of life. *Amœba* merely shares this power with all other masses of fluid. It is a suggestive fact, and one which has possibly a deep significance, that the chloroform drop (or other fluid) tends to take into itself especially such substances as will dissolve within it, or have a chemical affinity for it, just as *Amœba* tends to take within itself substances which it can digest.

The power of choice in inorganic fluids is bound up with the phenomena of solubility and surface tension, which cannot be discussed here.

These experiments on the power of choice in fluids are due to Rhumbler. There is absolutely no technical difficulty in performing them, and they are exceedingly striking and instructive.

4. *Other Activities Connected with the Taking of Food.* The method by which *Amœba* takes a small particle of food is very similar to that by which the chloroform drop takes within itself a bit of gum shellac. The protoplasm simply flows over and envelops the food particle. But at times the problem presented to the *Amœba* if food is to be obtained is much more difficult. Sometimes the food available is in the form of a long thread of *Alga*, many times the length of the *Amœba*. How is such an awkward piece of material to be managed? There seem to be only two possibilities for getting such a long thread into a short *Amœba*. One is to cut it into lengths, the other to coil it up. *Amœba* has no teeth for cutting up the thread, so it adopts the plan of coiling it. Individuals engaged in this process are sometimes found among the specimens studied in the laboratory; the process has been described in detail by Rhumbler.

The *Amœba* first settles itself upon the filament somewhere in its length, and envelops a portion of it. It stretches out a slight distance along the thread, then bends over, of course bending the filament at the same time. The bending is continued until there is a loop formed within the *Amœba*. The animal now continues to stretch out along the two ends and to bend them over, till the loop is doubled, tripled, and a coil is in process of formation. This is continued until the entire filament is rolled up into a neat little coil within the *Amœba*, where it is digested.

What are we to say to such a clever solution of a somewhat difficult problem as we have here? Must we admit to *Amœba* the power of grasping a situation

and intelligently adapting means to end to overcome difficulties—qualities which we are accustomed to consider as characteristic of minds in a high degree of development?

It will be well in this case as in others to test inorganic fluids before deciding what is to be thought of this matter. Suppose we present a similar problem to our chloroform drop; how will it meet the situation?

We may try the experiment as before, with a drop of chloroform at the bottom of a watch-glass of water. As the chloroform drop accepts hard shellac, we may present it with a bit of shellac drawn out into a long, fine thread, of length many times the diameter of the chloroform drop.

The chloroform envelops the filament in some portion of its length, just as *Amœba* did. Then it stretches out in both directions along the thread, exactly as was done by *Amœba*. (This is most striking when a drop of chloroform floating on the surface of the water is used, though the experiment is otherwise much more difficult to perform under these circumstances.) Thereupon the thread bends, exactly as with *Amœba*. The process now continues, precisely parallel with what occurs in the case of *Amœba* and the alga thread, until the shellac thread is coiled up within the chloroform drop, like a filament of *Oscillaria* within an *Amœba*.

This experiment is likewise due to Rhumbler. It is very easily performed; the following hints will be useful in doing so:

A drop of chloroform is placed with the medicine dropper on the bottom of a watch-glass full of water. The thread of shellac is obtained as follows: Two pieces of hard gum shellac are held with tweezers, one in each hand. The two are brought in contact, over a flame. The shellac is melted, so that the two pieces stick together. Now, by rapidly drawing them apart, fine threads, becoming hard at once, will be obtained. This operation will require some practice before it is done successfully.

The threads should be excessively fine, almost gossamer like, for the most striking results. Thick threads are wound up only very slowly.

In performing the experiment, it is important to bring the entire length of the thread completely beneath the surface of the water before letting it come in contact with the chloroform drop. If one end is allowed to project above the water, the surface film of the water resists the attempt of the chloroform drop to pull the thread down, so that no clear result will be obtained. Also, be very careful that there are no other minute drops of chloroform in the bottom of the watch-glass, as, if one of these comes in contact with the thread, it will pull the latter in the opposite direction from the main drop, and neither will succeed in rolling it up. The thread should lie freely in the water, in contact with nothing but the one drop of chloroform, if the best results are to be obtained. When the conditions are properly fulfilled, the experiment is a very striking one.

5. *Formation of "Artificial Diffugia Shells."* As is well known, *Diffugia*, one of the close relatives of *Amœba*, lives in a shell formed of sand grains, diatom shells, and other small particles cemented together. These particles are fitted together accurately in a single layer, so that no crevices can be discovered. How are these delicate houses built? It would seem that the process must

require much care, skill, and intelligence, to select the proper pieces and put them together with such nicety that the shell is but a single layer thick, and yet no gaps are left.

But the drop of chloroform is not to be outdone, and under the proper conditions will produce a shell not inferior to that of *Diffugia*. This may be shown very simply. Chloroform is rubbed up with fragments of glass in a mortar until the glass is reduced to the finest dust. Then with a pipette drawn out to a small point drops of this mixture of chloroform and glass dust are injected into water. At once the grains of glass come to the surface of the drops so formed and arrange themselves there in a single layer, without chinks or crevices, exactly as in the shell of *Diffugia*. The chloroform drop is covered with a shell of a delicacy and beauty equal to that of *Diffugia*, and almost indistinguishable in texture from it. Some of these artificial shells, if unexpectedly found with the microscope, would certainly be taken for those of *Diffugia*.

In place of chloroform, linseed oil or other oils may be used. They must then be injected into 70 per cent. alcohol, since the oil would float upon water. The process is exactly the same as when chloroform is used.

These experiments are likewise due to Rhumbler, who has so much extended our knowledge of the part played by physical laws in the activities of the lower organisms.

*Demonstration with the Projection Apparatus.* The experiments described above may be shown to individual students, or groups of students, or even perhaps performed by the students, if time permits. But the most satisfactory way of showing them to a large class is by projection on the screen. If a good stereopticon, employing the arc light, is available, this can usually be done in a satisfactory manner without other unusual apparatus. In the accounts usually given of projection with the microscope, much supplementary apparatus is described, which is unquestionably useful, but not necessary, so that the reader is likely to get the impression that projection with the microscope cannot be done without a complicated and expensive outfit. With a *good* stereopticon employing the arc light, a dish with parallel sides, full of water, to cut out the heat before the light strikes the concave mirror of the microscope to pass up through the lenses, any good microscope having low powers, and a mirror to place above the microscope, so as to reflect the images onto the screen, very satisfactory projection is possible. All the experiments described above may thus be shown to a large class, except the making of the "artificial *Diffugia* shells." The artificial shells may be shown on the screen after they are made, but even this is not very satisfactory, as the bits of glass of which the shell is made intercept so much light that the image of the shells on a screen is little more than a dark spot.

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## The Biology Laboratory in the Small High School.

This outline is intended for those small schools which do not have a laboratory. An endeavor has been made to suggest an equipment which will cost as little as possible, and yet be effective. The cheapest articles have not always been selected, because they frequently prove the most expensive. The teachers in these schools are overworked, and any cheap substitute which consumes time is not to be recommended. In such a school, a double nose-piece on a microscope saves, in a single year, more than its extra cost. The wages of the teacher should be taken into account, and anything which saves time and renders his work more effective is a wise expenditure. It is not good management to hire a man to work and fail to furnish him with the proper tools.

### THE LABORATORY ROOM.

A well lighted room, furnished with chairs and tables, should be provided. The cost is less than that of equipping with desks. The room may be used for other recitations if the science classes do not fully occupy the time. There is no reason why science work should be relegated to poorly lighted, heated, and ventilated basement or attic rooms. In fact, science work has greater need for light than other work. The laboratory is not a museum of curiosities, to be visited once or twice per week, weather permitting. It should be a place of activity and work, used every day and every hour of the day. It should, therefore, be as well lighted, heated, and ventilated, and as easy of access as any other room. If a school has but two rooms, one of these should be the laboratory. A fair laboratory can be made by replacing the rear seats of a large recitation room with chairs and tables. In small schools such an arrangement has some advantages. Double laboratory periods can seldom be secured, and pupils must necessarily do laboratory work at times when the teacher is hearing other recitations. The above arrangement keeps them in sight of the teacher, and ensures better work. If the chair legs have been rubber tipped the class in front will seldom be disturbed.

The building up of a laboratory depends largely on the preservation of material, and suitable case room is absolutely essential. Some provision must be made for water. A sink with running water should be readily accessible. If there is no system of city water works, a tank in the attic, which can be filled by a force pump, is the best substitute. It should be so constructed and so placed as to avoid freezing. It should be the janitor's duty to see that the tank is kept full. Large crocks make convenient receptacles for waste material. The janitor should empty these daily. If gas and Bunsen burners are not available, a supply of alcohol lamps and a blast lamp are necessary.

### GENERAL LABORATORY EQUIPMENT.

The following material and apparatus of a general nature should be provided. Most of it can be procured from local dealers, and is usable for other work than that of botany and zoölogy.

Tools and material: Hammer, saw, brace, bits, twist drills, flat file, triangular file, round file, screw driver, pliers, sand paper, nails, tacks, monkey-wrench, fine wire, screws, pins, thread, twine, sealing wax, wire netting, etc.

A work shop containing a suitable work bench will repay its cost yearly.

Carrying-trays, say 30 x 10 inches or 24 x 18 inches, for moving bottles, or for taking material to the basement to avoid freezing, should be made of thin wood and should have handles at the ends.

Window trays for holding plant boxes may be made of zinc, or wood lined with zinc. They should fit the window sills, and should be a trifle wider than the length of a chalk box. Three inches is a convenient depth.

#### INDIVIDUAL EQUIPMENT.

The school should provide each pupil with the following articles:

*A Dissecting Set.*—These cost less than one dollar per set, and will include, in a case, two mounted needles, one or two pairs of forceps, one scalpel, and one pair of scissors. The small lens sometimes included should be omitted.

*A Dissecting Microscope.*—These are so much more effective than the ordinary magnifying glasses, that the extra cost is more than compensated for. The Barnes dissecting microscope (Bausch & Lomb), with a doublet lens No. 2 (10 diameters), is a useful form. Other dealers have similar instruments. Cheap instruments with poor lenses should be avoided. An extra lens of higher power may be added when finances permit. Pupils should be encouraged, but not required, to purchase a pocket magnifier.

*A Dissecting Pan (for zoölogy work)*—Cheap substitutes may be made by the local tinner. Square glass candy trays are cheap and durable. Cork or wax bottoms are necessary.

The pupil should provide himself with the following:

*A Note Book.*—One with removable leaves is recommended; a portion of these should be drawing paper.

*A Drawing Pencil.*—Either HHH or HHHH quality.

*A Chalk Box.*—These are to be used for germinating seeds. Pine sawdust is better than sand.

#### APPARATUS.

The following equipment will enable the school to perform nearly all the experimental work in botany, zoölogy, and physiology given in any of the texts in common use. The quantities specified are such as experience has shown to be desirable for a class of twelve pupils. Pieces used but for a single experiment have been omitted.

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| 1 blast lamp.                                      | 1 nest beakers, assorted 1-4.  |
| 1 doz. dissecting microscopes (10 diam.).          | 1 doz. alcohol lamps, 4 oz.  |
| 1 doz. dissecting pans, paraffin bottoms.          | 1 doz. dissecting sets, including needles, forceps, scalpel, scissors. |
| 1 set cork borers (six).                           | 1 cork press.  |
| 1 doz. corks, large flat, $2\frac{1}{4}$ in. diam. | 1 gross assorted corks.  |
| 3 graduates, 100 c.c., cylindrical.                | 2 doz. rubber stoppers, single and double perforated.                  |
| 1 set weights, 2 kilo to 5 grams.                  |  |

1 Harvard trip balance.	12 feet rubber tubing, $\frac{1}{4}$ in.
1 mortar, iron, quart.	$\frac{1}{2}$ gross test tubes, 4 in.
1 mortar, porcelain, $3\frac{1}{4}$ in. diam.	2 doz. dropping bottles.
1 doz. beakers, $1\frac{1}{2}$ oz.	$\frac{1}{2}$ doz. funnel tubes.
2 funnels, 2 in., 4 in.	$\frac{1}{2}$ doz. battery jars, 4 quart.
1 doz. test-tube brushes.	$\frac{3}{4}$ ring stands, 3 rings.
2 doz. test-tubes, assorted.	$\frac{1}{2}$ doz. thermometers.
$\frac{1}{2}$ doz. battery jars, 1 quart.	1 lb. glass tubing, $\frac{1}{4}$ in.
1 doz. watch crystals, Syracuse.	2 doz. bottles (vaseline bottles may be substituted).
4 packages filter paper, 2 of 3 in. and 2 of 6 in.	

The above list will cost about \$75.00.

Other apparatus, as follows, will be needed, but it can probably be secured of local dealers at a total cost of about \$5.00.

- 1 doz. jelly tumblers with covers. Those with perpendicular sides are preferable. Stender dishes are better, but more expensive.
- 2 doz. large, clear glass tumblers.
- 2 doz. fruit jars, mostly pints and quarts.
- 1 doz. soup plates.

#### CHEMICALS.

Iodine crystals, - - - 10 g.	Mercury, - - - - 1 lb.
Potassium iodide, - - - 25 g.	Eosin, - - - - 100 grams.
Ether, commercial, - - 250 g.	Alcohol, 95 per cent. - 1 gal.
Nitric acid, conc., - - 4 oz.	Alcohol, wood, - - 1 gal.
Sulphuric acid, conc., - 4 oz.	Alcohol, absolute, - 100 g.
Hydrochloric acid, concentrated, - - - 4 oz.	Caustic potash, pure, - 250 g.
Ammonium hydrate, - - 4 oz.	Potassium chlorate, - 250 g.
Four bottles for above with names blown on bottles.	Formalin, - - - - $\frac{1}{2}$ gal.
	Glycerin, - - - - 50 g.
	Chloroform, - - - - 2 oz.

Total cost, about \$10.75.

Other chemicals, grafting wax, cotton seed oil, lime water, materials for nutrient solution, seeds and material, can be secured locally. Fehling's solution should be made as needed. A small amount of money should be in the hands of the teacher, to be used for this purpose.

#### LIBRARY.

The school should provide at least a few reference books. The following are desirable:

Bailey.	Botany; an elementary text-book.—Macmillan.
Coulter.	Plants.—Appleton.
Ganong.	Plant Physiology.—Holt.
Bergen.	Foundations of Botany.—Ginn & Co.
Atkinson.	Elementary Botany.—Holt.
Newell.	Outlines of Lessons in Botany, two parts.—Ginn.
Barnes.	Plant Life.—Holt.
Jordan & Kellogg.	Animal Life.—Appleton.
Davenport.	Introduction to Zoölogy.—Macmillan.
Herrick.	The Home Life of Wild Birds.—Putnam.

- Comstock. Insect Life; Teachers' and Students' edition.—Appleton.  
 Holland. The Butterfly Book.—Doubleday & McClure.  
 Total cost, about \$16.50.

For an additional list of books see Handbook for Teachers, to accompany Bergen's Foundations of Botany; Atkinson's Elementary Botany, Appendix; School Review, November, 1900, page 563; Teachers' College Record, Vol. I, No. 2, page 85. Every teacher should have Ganong's Teaching Botanist, Macmillan; the chapter on Botanical Books and Their Uses contains much valuable information. Kerner & Oliver's Natural History of Plants, in four volumes (listed at \$15.00, Holt), is excellent, and should be procured if possible.

### THE MICROSCOPE.

In schools where but one compound microscope can be provided it is well to have a fairly good one. A desirable instrument is the Bausch & Lomb BB4, with two-inch and one-inch eyepieces, and two-thirds and one-sixth objectives and case. Similar equipment can be secured in other standard makes, such as Zeiss, Leitz, and Reichert. A B2, with one eye-piece, two-thirds and one-sixth objectives, double nose-piece and case, will cost about ten dollars less. Microscopes and some other scientific apparatus for school use can be imported duty free.

The kind and quantity of microscopical work attempted will determine the amount of necessary equipment. The following is suggested:

$\frac{1}{2}$ gross slides.				
1 oz. cover-glasses, $\frac{3}{4}$ inch square.				
3 culture slides.				
1 razor.				
1 oil stone.				
Xylol, C P in bottle,	-	-	-	250 g.
Paraffin, soft, melting point 45°,	-	-	-	500 g.
Paraffin, hard, melting point 52°,	-	-	-	500 g.
Hæmatoxylin (Delafield's),	-	-	-	100 g.
Grenacher's borax carmin,	-	-	-	100 g.
Picro carmin stain,	-	-	-	100 g.
Methyl green (powder),	-	-	-	10 g.
Canada balsam, pure,	-	-	-	20 c. c. tube.
Picro sulphuric acid,	-	-	-	100 g.
Chromic acid, crystals,	-	-	-	25 g.
Corrosive sublimate,	-	-	-	25 g.
Total cost, about \$6.00.				

### AUXILIARY APPARATUS.

Many other things are convenient, but not indispensable. It may be well to name a few of these:

One glass graduate, 1000 c. c.,	-	-	\$ 2.15
An aquarium (very desirable),	-	\$2.00 to	12.50
Two large bell jars,	-	-	2.60
A Wardian case,	-	-	13.50
Mounted slides,	-	\$5.00 to	12.00
Bromide enlargements of photo-micrographs, each,	-	-	2.50

One chemical thermometer, C.200,	-	-	\$ 1.10
A microtome,	-	-	\$5.40 to 20.00
Several low bell-jars, each,	-	-	.50
Syringe and cannula,	-	-	7.50
Several preserving jars of various sizes.			

See Bergen's Handbook and Ganong's Teaching Botanist for further description of auxiliary apparatus.

### SUBSTITUTIONS.

It will be seen that the above lists may be varied considerably without detriment. The individual experiences of teachers will suggest desirable substitutions and omissions. These may be either in the line of securing better and more costly apparatus, or the procuring of something less expensive. Some of these latter are suggested. Twist drills may serve fairly well as cork borers. The dissecting tools may be wrapped in flannel; this will avoid the rusting which comes from putting damp instruments into the case. Cheap lenses should never be substituted for good ones. Dissecting pans are not so much used as formerly; some teachers perform much of the work on oilcloth, and then use any available dish when it is desirable to have the dissection under water. Chalk boxes for germinating experiments are suggested because of their cheapness; dampness is liable to warp them and cause trouble. Small thumb pots are very cheap, and make desirable substitutes. Tin cans with the tops melted off and the bottoms perforated for drainage, may be used. A tin pan filled with sand may be heated by a single flame, and will serve several pupils. It will be convenient to have all bottles and flasks with uniform sized openings, and rubber stoppers to fit these, thus making them interchangeable. For the same reason only one size of glass tubing should be purchased. Beakers are easily broken. Tinware or agateware may be substituted. Change of temperature causes the bottoms of battery jars to crack. Small aquaria may be secured direct from the glass companies at nearly the same cost. Jewelers frequently have old watch crystals which can be had for the asking. Quinine bottles are excellent for collecting and other purposes. If used with cyanide, the teacher should prepare them for use. This may be done by covering the cyanide with powdered lime or plaster of Paris and then moistening it. Rubber cloth, or even oilcloth, may be used instead of sheet rubber in transpiration experiments. Collecting nets, water nets, setting boards, and many other things can be made by the class. Small broom straws dipped in melted paraffin will serve the purpose of bristles.

### COST.

The total cost of material, exclusive of the laboratory tables, chairs, and general equipment, is given below. This amount will vary according to the number in the class.

1 compound microscope,	-	\$37.50	Apparatus as given,	-	-	\$75.00
12 dissecting microscopes,	-	22.50	Chemicals as given,	-	-	10.00
12 dissecting sets,	-	10.25	Microscope accessories as given,	-	-	6.00
12 dissecting pans,	-	4.90	Reserve fund for running ex-	-	-	
Library,	-	20.00	penses,	-	-	15.00
Total cost,						\$201.15

If impossible to complete the equipment the first year, it is suggested that the school board appropriate one hundred dollars the first year, and fifty dollars for two succeeding years. In this case the reduction should be made in the number of pieces, not in the quality of them. The hundred dollars may well be expended as follows:

1 compound microscope, -	\$37.50	Microscope accessories, -	\$ 5.00
12 dissecting microscopes, -	22.50	Reserve fund, - - -	5.00
12 dissecting sets, - - -	10.25	Apparatus, - - - -	14.75
Chemicals, - - - - -	5.00		
			<hr/> \$100.00

The second and third items are especially important, for it is with these that the pupil performs most of his work, and it is very desirable that each pupil have these.

#### LIST OF DEALERS.

Alfred Robbins & Co., Chicago, Ill.  
 Bausch & Lomb Optical Co., Rochester, N. Y.  
 Cambridge Botanical Supply Co., Cambridge, Mass.  
 Central School Supply Co., Chicago, Ill.  
 Chicago Laboratory Supply and Scale Co., Chicago, Ill.  
 Eberbach & Son, Ann Arbor, Mich.  
 Eimer & Amend, New York City.  
 Knott Scientific Apparatus Co., Boston, Mass.  
 Queen & Co., Philadelphia, Pa.  
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 Sargent & Co., Chicago, Ill.

University of Illinois.

STRATTON D. BROOKS.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### I.

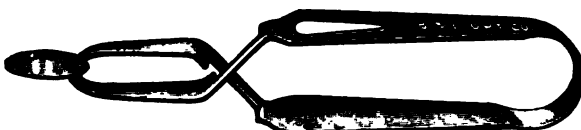
These notes and suggestions on Medical Micro-Technique have been prepared at the suggestion of many workers with the microscope who find need for a reference work free from the mass of detail found in books that treat both of methods and diagnosis. The most reliable methods are described briefly and concisely so that the beginner and the busy practitioner may get the greatest assistance possible from the use of his microscope. There will be a constant repetition of the simplest directions, which seems unavoidable, as it is desired to have each subject complete without referring to other portions for technique that may be common to a number of different methods.

For more detailed descriptions and other methods, the reader is referred to more pretentious works.

I am indebted to Dr. Adolph Gehrmann and Dr. W. A. Evans for valuable suggestions and for the loan of some of the preparations photographed.

## PREPARATION OF BACTERIA.

Preparations of bacteria for examination under the microscope are, in the main, easy to make. The apparatus needed is simple and easy to manipulate. A pair of Cornet forceps, a pair of plain cover-glass forceps, an inoculator, consisting of a glass rod about eight inches long with two and one-half inches of No. 27 gauge platinum wire sealed into one end of the rod, some clean covers, slips, staining solutions, Canada balsam, and a Bunsen burner or alcohol lamp are about all that will be required.



Cornet's Forceps.

To make a mounted preparation, the platinum wire in the inoculating rod should be bent into a small loop at the end. It should then be heated over the alcohol or Bunsen flame until it is red hot, to sterilize it. A little of the material to be examined should be taken up by this loop and spread thinly on a cover-glass held in a Cornet forceps. Before laying down the inoculator it should be reheated to sterilize it and prevent the carrying of infection. Allow the thin film of material on the cover-glass to dry in the air, then pass it through the flame of the alcohol lamp or Bunsen burner three times rapidly, being careful to keep the film surface upward. It is now ready for staining, and may be stained with almost any of the anilin dyes. The stains most generally used are Loeffler's alkaline methylen blue, gentian violet, and methyl violet. These stains may be purchased ready for use or may be prepared according to the following formulæ:



Cover-glass Forceps.

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Inoculating Needle.

## LOEFFLER'S ALKALINE METHYLEN BLUE.

Concentrated alcoholic solution of methylen blue	-	30 c. c.
Caustic potash, one per cent. solution	-	1 c. c.
Distilled water	-	100 c. c.

## GENTIAN VIOLET.

Gentian violet, saturated alcoholic solution	-	11 c. c.
Absolute alcohol	-	10 c. c.
Anilin oil, clear three per cent. in distilled water	-	100 c. c.

## METHYL VIOLET.

Methyl violet	-	1 gram.
Dissolved in absolute alcohol	-	20 c. c.
Anilin water	-	80 c. c.

Make the anilin water for either formula as follows: Shake up three c. c. of clear anilin oil in 100 c. c. of distilled water. Shake thoroughly several times and filter till it is clear, when it is ready for use. These stains are applied to the

cover-glass with a pipette until the smeared surface is entirely covered. Allow the blue to stain from one to five minutes, but the methyl or gentian violet will ordinarily require but a few seconds. The time required for staining can be quickly determined by a few trial preparations. Wash the cover-glass thoroughly in water, absorbing all excess of water by blotting it between two pieces of filter paper, dry it in the air and mount it on the slide, film side down, in a small drop of Canada balsam. The preparation is now ready for examination under the microscope. It should be first examined with the 2-3 and 1-6 inch objectives and later with the 1-12 oil immersion. The above instructions apply to bacteria in general. Some bacteria, however, are exceedingly difficult to stain, but when once stained are exceedingly difficult to decolorize, and upon these peculiarities recognition of certain forms is largely based.

Harvey Medical College.

WILLIAM H. KNAP.

## MICRO-CHEMICAL ANALYSIS.

### XIX.

#### THE COMMON METALS.—SILVER GROUP.

As a matter of convenience we can consider that this group comprises, as in qualitative analysis in the wet way, the elements, silver, mercury in the form of mercurous salts, lead, and the rare element thallium in the thallos condition.

These elements, though widely separated in the Periodic System, are thus grouped together because of the fact that their chlorides are of low solubility, and are therefore precipitated more or less completely by dilute hydrochloric acid or by solutions of chlorides. The other halogen acids and their salts also yield precipitates with the members of this group.

Because of their being precipitated by dilute hydrochloric acid, the micro-chemical separation of the silver group from other elements would seem to be a very simple process; this, however, is not always the case, since there is not infrequently a tendency toward the formation of double salts, etc., which tends to complicate matters.

Antimony and bismuth under certain conditions can be precipitated as antimonyl chloride— $\text{SbOCl}$ —and bismuthyl chloride— $\text{BiOCl}$ ; more rarely copper may separate as cuprous chloride— $\text{CuCl}$ . In all three cases the precipitate is soluble in a slight excess of the reagent. It must also be remembered in this connection that concentrated hydrochloric acid will cause the separation of not a few salts by forcing back their dissociation. Moreover, the addition of hydrochloric acid to alkaline solutions may often produce a precipitate of some compound held in solution by the alkali.

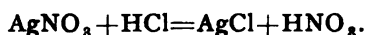
#### SILVER.

Of the many reagents which may be employed for the detection of silver, the following will be found to answer all requirements:

- I. Hydrochloric Acid.
- II. Ammonium Dichromate.
- III. Arsenic Acid.

In addition to the crystalline salts given by the above reagents, the worker should become familiar with the appearance of the precipitates formed by a number of other reagents when brought in contact with silver salts. Chief among these may be placed oxalic acid, primary sodium tartrate, primary sodium phosphate, potassium antimonyl tartrate, sodium acetate, sodium sulphate, and ammonium carbonate; most of these silver compounds are of no real or actual value so far as analytical work is concerned, but most of them are of considerable interest from a theoretical point of view, and because of the fact that in the majority of cases excellent crystals, for crystallographic study with the microscope, can be obtained; for this reason it has been thought wise to devote a little space to their consideration.

*I. Hydrochloric Acid added to solutions of salts of Silver, precipitates Silver Chloride.*



*Method.*—Acidify the clear test drop with nitric acid, then, at the center, add a drop of dilute hydrochloric acid. If silver is present an immediate precipitate should result. Examine under the microscope. Silver chloride is so insoluble in water that it is thrown down as an amorphous mass. If the precipitate is wholly crystalline, either silver is absent or else present in very small amount. In order to identify silver in an amorphous precipitate it is necessary to recrystallize it. Before so doing it is always advisable, and often necessary, to first remove the solution from the precipitate and wash the latter. If the hydrochloric acid has been carefully added and the drop not stirred, it is easy to draw off the clear solution from the curdy, heavy precipitate of silver chloride. When the amount of precipitate is very small it is best to have recourse to the centrifuge to accomplish the separation. After removing the supernatant liquor, wash the precipitate once or twice with hot water acidified with nitric acid. The washed precipitate is then recrystallized from (A) concentrated hydrochloric acid, or (B) ammonium hydroxide.

A. To the precipitate of silver chloride, at the corner of a slide, add a drop or two of concentrated hydrochloric acid, and heat the preparation over the "micro" flame. If the precipitate is not completely dissolved, draw off rapidly the hot acid, without exercising any great care, to obtain a perfectly clear drop. On cooling, tiny crystals of silver chloride separate (Fig. 75). Octahedral crystals predominate.

B. To the washed precipitate add one or two drops of strong ammonium hydroxide. After a second or two of contact, draw off the ammoniacal solution from any undissolved precipitate. Do not heat the preparation. Allow the preparation to stand. Almost immediately the drop becomes turbid around the edges, because of the separation of minute crystals of silver chloride; these crystals increase slowly in size, but are always very small, requiring a moderately high power for distinguishing their form. From ammoniacal solutions silver

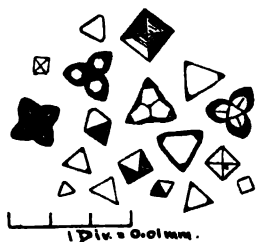


Fig. 75.

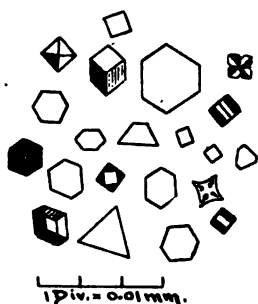


Fig. 76.

chloride seems to separate almost invariably in the form of cubes and hexagonal and rectangular plates (Fig. 76). More rarely octahedral crystals are obtained.

Of the two recrystallization methods, that with ammonium hydroxide will be found to be the better, as well as also the more convenient, because of the greater solubility of the precipitate in this reagent, and because the employment of ammonium hydroxide eliminates many interfering substances.

*Remarks.*—When working with concentrated hydrochloric acid or strong ammonia, great care must be used to avoid spoiling the microscope and objectives. It is essential to work rapidly.

The drop is acidified with nitric acid because the presence of this reagent favors the agglutination of the particles of silver chloride, and hinders at the same time the precipitation of oxychlorides, etc.

Decanting after precipitation is advisable, since it has been shown by a number of investigators that the crystal form of silver chloride is changed by many compounds when the former is crystallized in the presence of the latter. Still other compounds completely ruin the test. Although there is, of course, danger of the occlusion of some of these objectionable salts by the silver chloride, this difficulty is reduced to a minimum by avoiding too concentrated test drops and washing the precipitate.

Washing the precipitated silver chloride with hot water removes the greater part of the lead chloride which may have been carried down with the silver.

Treated with ammonium hydroxide, silver chloride dissolves with the formation of the compound  $\text{AgCl} \cdot 2\text{NH}_3$  (Isambert). If mercurous chloride is present the precipitate turns black under the action of the reagent; an insoluble compound being formed which Barfoed has shown to be a mixture of metallic mercury and the compound  $\text{Hg} \cdot \text{NH}_2 \cdot \text{Cl}$ . If, therefore, silver chloride is present only in traces in a precipitate consisting chiefly of mercurous chloride, ammonium hydroxide may dissolve practically no silver chloride, since the finely divided metallic mercury may reduce the greater part of the silver salt to metallic silver. Under such conditions it is necessary to exercise the greatest care in order to avoid missing the little silver which is present.

Thallous chloride is precipitated by hydrochloric acid in the form of cubes and stars.

#### *Exercises for Practice.*

Precipitate with dilute  $\text{HCl}$  a test drop containing  $\text{AgNO}_3$ . Separate and wash the precipitate; then recrystallize it by the above described method (A), using concentrated  $\text{HCl}$ . Then repeat the experiment, using  $\text{NH}_4\text{OH}$  as the solvent (B).

Make a mixture of  $\text{Ag}$  and  $\text{Pb}$ , test by both recrystallization methods.

In like manner test a mixture of  $\text{AgNO}_3$  and  $\text{HgNO}_3$ .

Precipitate with  $\text{HCl}$  a test drop containing  $\text{Pb}$  and  $\text{Ag}$ ; recrystallize the precipitate without drawing off the solution. In like manner test mixtures  $\text{Ag}$  and  $\text{Zn}$ ,  $\text{Ag}$  and  $\text{Cd}$ ,  $\text{Ag}$  and  $\text{Sb}$ ,  $\text{Ag}$  and  $\text{Bi}$ ,  $\text{Ag}$  and  $\text{Pt}$ ,  $\text{Ag}$  and  $\text{Sn}$ ,  $\text{Ag}$  and  $\text{Cu}$ .

Try recrystallization of the silver chloride in the presence of phosphates, in the presence of sulphates, in the presence of molybdates.

Cornell University.

E. M. CHAMOT.

## NOTES ON TECHNIQUE, I-II.\*

I. *The Preparation of Sections of Hydra for Class Work.*

The preparation of satisfactory sections of Hydra for microscopical study of the tissues has always been attended with more or less difficulty. Usually the cell boundaries are poorly defined, sub-epithelial cells are not clearly marked off, and on account of the presence of large quantities of metabolic products—fat globules, etc.—in the cells, the sections as a whole have an indefinite, crowded appearance. After some experimentation the following method of preparing and staining sections of Hydra was tried with excellent success. Its results are very satisfactory; cell boundaries are clearly marked, so that sub-epithelial cells may readily be seen by the student, the muscle processes appear with entire distinctness, and finally the cytological relations of the cells are well defined.

The method used is in detail as follows: Large specimens of the common brown Hydra (*H. fusca*) are placed in Stender dishes filled with filtered tap water and allowed to "starve." The purpose of this "starving" process is to rid the cells of stored-up fat and other metabolic products. Since the animals are placed in dishes containing only clear water they are unable to feed and so use up gradually these reserve and intermediate products of assimilation. This "starving" is perhaps the most important step in the process, as on its thoroughness the character of the sections depends. The length of time which must be allowed for the animals to become entirely free of fat globules will depend on their condition at the beginning. We have found in practice that from one to two weeks is usually a sufficient length of time. When the process is completed the animals are very light, in fact almost white, in color and are more than ordinarily transparent when fully extended. Some of the specimens may die in the course of the "starving" process, but not any considerable number are lost in this way.

When thoroughly "starved" the animals are fixed in vom Rath's picro-osmo-aceto platinic chloride mixture<sup>1</sup> for about an hour, then washed in methyl alcohol, blackened for several hours in pyroligneous acid, again washed in methyl alcohol, removed to 70 per cent. alcohol and carried up through 96 per cent. and absolute alcohol, cleared in cedar oil, and embedded in paraffin in the ordinary way.

The animals are killed almost completely extended in the following way. A Hydra isolated in a pipette with a small amount of water is placed on a slide or

\* These "Notes" do not make any claim for originality, but are merely detailed accounts of some applications of ordinary methods which have been found in practice to accomplish in a satisfactory way the desired ends. They are offered in the hope that they may prove useful to teachers. R. P.

<sup>1</sup> vom Rath, O. Zur Conservirungstechnik. Anat. Anz. II: 280-288, 1895.

Formula (p. 283):

	200 c. cm. saturated aqueous solution of picric acid.
Added in	{ 25 c. cm. 2 per cent. aqueous solution of osmic acid. 1 g. platinic chloride, dissolved in 10 c. cm. of water. 2 c. cm. glacial acetic acid.
the order	
given.	

Fixation in Hermann's fluid, with subsequent treatment with pyroligneous acid in the same way, has been found to give equally good results.

in the bottom of a watch-glass with a drop of water just large enough to cover it. If then placed in fairly strong light, as on the stage of a microscope over the opening of the condenser, the animal will soon expand to its full length. Then

from a pipette filled with the vom Rath mixture a stream of this strong fixing agent is suddenly and forcibly thrown on the Hydra. The specimen will, provided the stream is directed so as to strike it squarely and is sent with some force, in nearly every case be killed before it contracts to any extent.

Sections are cut five or ten mikrons in thickness, fastened to the slide with Mayer's albumen fixative, and stained by the Heidenhain iron-haematoxylin method.

FIG. 1.—Photograph of portion of cross section of Hydra showing character of preparation made by the method described. The photograph is much less clear and precise in definition than the preparation. Along the upper left side of the figure the muscle processes may be distinctly seen just at the inner ends of the ectoderm cells.

od. The sections are mordanted about thirty minutes in the iron alum (two per cent. solution), *thoroughly* washed in water, stained for thirty minutes in a one-half per cent. aqueous solution of haematoxylin, washed again, and differentiated in the iron alum solution. The differentiation should be continued till only the nuclei, cell boundary regions, and muscle processes are stained. No counterstain is used. The sections are then carried up through the different grades of alcohol, cleared in xylol, and mounted in balsam.

The resulting sections are almost diagrammatic in their clearness and are very satisfactory for class use. Such things as the muscle processes, which are ordinarily so difficult of demonstration to students, are so clearly brought out as to cause no trouble whatever. We have appended to this paper a photograph showing, in some degree, the character of the sections prepared in the way just described.

RAYMOND PEARL AND LEWIS H. WELD.

## II. *The Demonstration of Nerve Fibers in the Ventral Cord of the Earthworm.*

The following method of making sections of the ventral nerve cord of the earthworm has been found to give preparations which furnish very clear pictures of the structure of nerve cells and their associated axis cylinder processes. The plan was developed with the idea of bringing more forcibly to the student's mind than seems possible with the conventional cross section an adequate conception of the most important structural elements of the nervous system, viz., the ganglion cells.

The preparations are made in the following way: Pieces of the ventral cord one and one-half to three centimeters in length are dissected out of a freshly killed worm in physiological salt solution. These pieces are then stretched in small, wax bottomed dissecting pans and held in a straight position by means of

small pins at either end and the lateral nerves are laid out in their normal positions. The salt solution is then poured off, leaving the pieces of cord straight and flat against the bottom of the pan. A considerable quantity of vom Rath's fluid (formula given above) is now poured into the pan and the tissue is left in this for two hours for thorough fixation. In a short time (fifteen to twenty minutes) after this fixation has begun the pins may be removed, and the pieces of cord handled freely without danger of distortion. After removal from the fixing fluid the tissue is washed in methyl alcohol; blackened in pyroligneous acid from eight to twelve hours, and washed again in methyl alcohol. The pieces may now be dehydrated in 96 per cent. and absolute alcohol, cleared in cedar oil and embedded in paraffin.

Pieces of the cord from three-fourths to one and one-half centimeters long prepared in the way described are cut into longitudinal sections twenty mikrons thick. The plane of cutting is made as nearly as possible horizontal, i. e., at right angles to the median dorso-ventral plane of the cord. The sections are then stained on the slide by the Heidenhain iron-hæmatoxylin method and then differentiated in a two per cent. iron alum solution till the axis cylinder processes alone retain the color. The sections are faintly counterstained with orange G and, after dehydration and clearing, mounted in balsam.

The resulting preparations show the nerve fibers stained an intense black on a light yellow field, and as a result of the great thickness and the plane of cutting of the sections, a single fiber may be followed along the cord for a long distance. For precision and distinctness in the differentiation of fiber elements these preparations leave little to be desired, and the simplicity of the process as compared with other nerve methods is a decided recommendation.

Zoological Laboratory, University of Michigan.

RAYMOND PEARL.

## A Convenient Case for Butterflies and Moths.

Many enthusiastic collectors of butterflies and moths have been discouraged by having their specimens destroyed by museum pests.

In order that a collection may be of greatest value it must be so arranged that the specimens may be readily examined and at the same time so arranged that they may be permanently preserved. Many attempts have been made to accomplish this.

The Harvard cabinet fulfills the first requirement, but it is not wholly satisfactory as regards the second. Furthermore, in examining the specimens they are sooner or later badly damaged. Especially is this the case when the specimens are used in class work.

The Denton Brothers' Butterfly Tablets come more nearly meeting the requirements. In this case the specimen is sealed up between a plaster cast and a plate of glass. This makes a compact case and readily handled without danger to the specimen. A very serious objection, however, is to be raised to this method of preserving, which is that only one side of the specimen can be

seen. As is well known, an examination of both dorsal and ventral sides of a butterfly is frequently necessary and is always desirable.

With this thought in mind, the writer has attempted to contrive a case which shall meet all the requirements. It consists of a flat box about one inch deep whose edges are composed of wood, and whose sides are plates of glass. Between these plates of glass the insect is supported by means of a pin attached to one glass by a bit of sealing wax.

Figs. 1 and 2 are photographs of the dorsal

FIG. 1. Dorsal View.

and ventral views of a specimen in such a case. A detailed description is as follows:

Figure 3 represents a diagrammatic section through the case and through the insect (i). The frame (ff) is composed of wood rabbeted to receive the two glasses (gg). These glasses are fastened in place permanently by putty (a). The pin (p) which supports the insect is passed through a small piece of cork (c) before it is inserted into the insect. This cork is attached to the glass by a very small quantity of sealing wax and thus forms a larger base for support. A much better arrangement would be to have a large flat head on the pin, in which case the cork could be dispensed with.

Figure 4-A represents a partial cross section of the case when pressboard or heavy cardboard (cb) is used for the framework. The pieces of glass (gg) are fastened by pasting paper (p) over as at (f). The paper is represented as still unfolded (pi). Fig. 4, B and C represent a cross section and longitudinal view of the pressboard framework and binding paper. The pressboard is cut on the dotted lines so as to readily fold to form the corners. The distance between the

FIG. 2.—Ventral View.

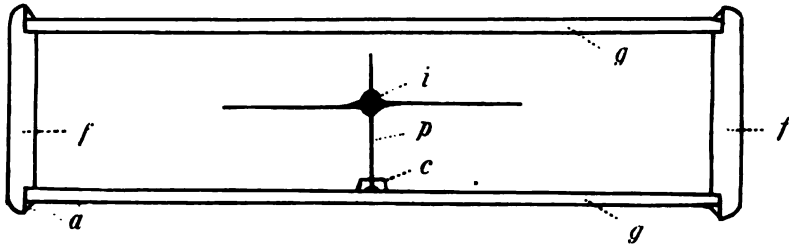


FIG. 3.

two glasses will depend on the size of the insect and the length of the pin. In many cases it would not need to be more than half an inch.

It is scarcely necessary to say that the insect should be pressed and dried before mounting. The supporting pin is inserted into the insect when it is spread for drying. I find it best to insert it from the ventral side. Great care should be exercised in making the pin stand perpendicular to the body. As I dry the insects with their wings at right angles to the body, a smooth flat board is all that is necessary for a drying board. By means of the supporting pin the insect is fastened to the drying board, with its ventral aspect uppermost. This allows careful arrangement of legs, antennæ, and evisceration when necessary,

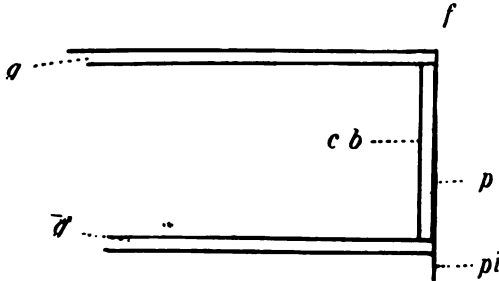


FIG. 4-A.

and in no way hinders the proper arrangement of the wings.

The case may be all completed with the exception of putting in the glass to which the insect is attached. This is done after the insect has been thoroughly dried and carefully attached to the glass by the supporting pin.

When the insect is completely mounted the only thing which will obstruct the view any whatever will be the bit of cork or the flat head of the pin, which need not be at most more than a quarter of an inch in diameter. All parts of the insect can be readily examined even with a hand lens, while at the same time it is permanently and securely sealed from all insect pests.

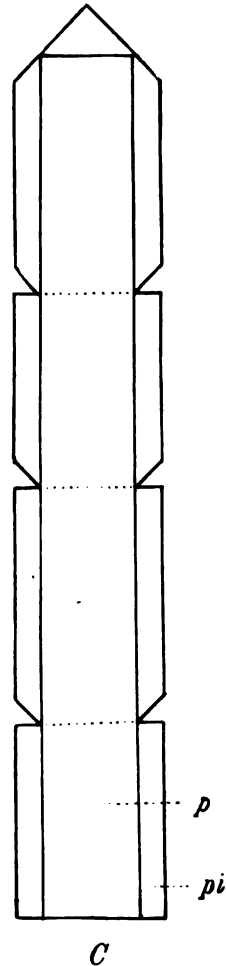
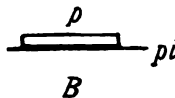


Fig. 4.

## LABORATORY PHOTOGRAPHY.

*Devoted to Methods and Apparatus for Converting an Object into an Illustration.*

### PHOTOMICROGRAPHY.

#### III. Illuminating the Object.

Before any photograph is attempted the optical parts shown in Fig. 1 (see cut, page 1525, JOURNAL OF APPLIED MICROSCOPY, November, 1901) must be centered. These parts are the condensers, the diaphragms, and the microscope. They are sufficiently centered if circular cardboards cut from dark pasteboard and pierced with a pin hole in the center, and fitted into each one of them, per-

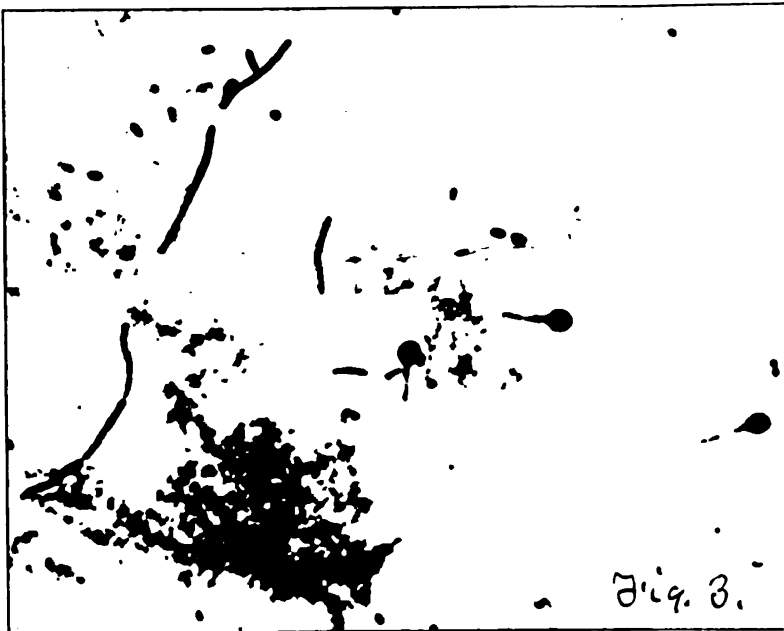
Golgi brain cell of the first type, from the hippocampus of a cat; the small black lines of the dendrons and neuraxon would be drowned by too large a diaphragm and doubled by one too small. The figure was made with a 16 mm. apochromatic objective and a camera extension of forty-seven inches without eyepiece. x 70.

mit a ray of light to pass through all to the center of the cross on the ground glass. These cardboards can be kept, and the centering can be quickly tested from time to time.

The light and condensers, including the Abbe, should be so arranged that the image of the source of light coincides with the object to be photographed, if a 16 mm. objective or one of higher power is to be used. If a thin paper is placed under the clips on top of a slide, the image can be seen; perhaps a surer way is

to focus a low power lens exactly on an object so that it is distinctly seen on the ground glass; then remove the object and so adjust the light and condensers that an image of the source of light is sharp on the ground glass; then put on high power objective desired and the instrument is ready for use. Various arrangements of the condensers and light will produce this image; and the image will vary in size with the several arrangements; some particular arrangement will be found best for each objective, or at least for each group of objectives.

In doing miscellaneous work it will often become necessary to change from one arrangement to another. For this reason the rods carrying the light and the condensers should be so graduated that permanent records of each arrangement can be made. This is of such importance as a time saver that I think it



*Bacillus tetani* in both the vegetative and spore forming conditions. x 1500.

should have the attention of instrument makers. We experimented for months with our instrument to little or no purpose; this we afterward found arose from the fact that nearly right was entirely wrong. Several evenings would be wasted in getting a correct arrangement; we could then work rapidly while we required the same arrangement; but to break it up and return to it again was almost the same trouble it was to find it at first; our real progress began when we had carefully graduated both tables so that the Abbe, the double convex lens, the condenser with its adjustable front and the light, five variables, could be brought quickly into any desired relationship.

The image of the luminous portion of the light must of course be as large as the required field, or the above arrangement will not do; accordingly, for low power work the image of the light must fall on the objective instead of the object.

For this class of work it is therefore necessary to make a special arrangement and record it.

The field to be taken must of course be evenly illuminated; this will be so nearly secured by the arrangements mentioned above that it can be completed by looking the field over on the ground glass and slightly moving the light.

The best work requires the illumination of the object by light of nearly the same wave length. This is easily accomplished by the use of Zettnow's filter. Nearly all photographic houses sell screens of some sort; a lantern slide fixed without exposure and stained with tropæolin is recommended; we have tried several of these substitutes, but find nothing better than Zettnow.

The solution is prepared as follows: 160 grams—pure dry copper nitrate; 14 grams—pure chromic acid; water to make up 250 c. c. The color screen should be one cm. thick.

In the illumination of the object it is essential that the cone of light bear the proper relationship to the numerical aperture of the objective if refraction and not color absorption is to produce the image. A cone too wide will dim the object (fog the negative) or in some cases entirely drown it. A cone too narrow will in all cases utterly ruin it with diffraction halos. This fault is the commonest in photomicrography for the reason that a small cone of light minimizes focus difference and gives a sharp appearance to all parts of the object on the ground glass. This appearance is, however, not a faithful picture of the object.

The cone of illumination should be equal to the cone entering the objective; the wider therefore its angular aperture the wider the diaphragms should be opened. The best practical ways I know to ascertain the proper diaphragm in a given case, is to take out the eyepiece and, looking into the microscope tube, reduce the diaphragm until a bright circle of light fills about one-third of the objective. Another way is to look in without removing the eyepiece through a hand lens such as one uses in focusing on the ground glass. The light must of course be reduced with ground glasses or color screens before one ventures to look at it in either of these ways.

In the photography of colored objects, as bacteria (Fig. 3), in which absorption in different degrees produces the image, the diaphragm may without detriment be opened wide.

D. W. DENNIS.

Earlham College.

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The Palasade Mfg. Co., of Yonkers, New York, have issued two small pamphlets for free distribution among physicians, which may be found useful. One is entitled "The Essentials of Hematology" and contains careful directions for the study of blood in the detection of disease. The second is "A Syllabus of Bacteriology." This is a simple account of the facts of bacteriology which bear on practical medicine, together with descriptions of methods of bacterial study. The work is clear, is illustrated by rather gorgeous colored plates, and will be found useful to physicians who are interested in developing the practical side of bacteriological technique. Either of these pamphlets may be obtained from the publishers.

H. W. C.

## NOTES ON THE MICROSCOPE.

### I. Early Microscopes.

These notes are offered with no pretense to completeness or originality. If they give usable information regarding the origin, development, construction, use, and care of the microscope to any who do not have it, they will have fulfilled the purpose for which they were intended.

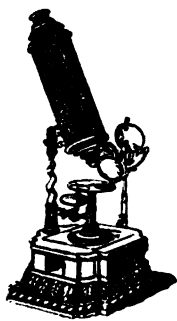
Lenses have been known from very early times, lens-shaped pieces of rock crystal having been made as early as 720 B. C. Although simple magnifying lenses were used as such in 1276 by Roger Bacon, a monk, it was not until about 1590 that the first combination of lenses into what might be termed a crude microscope was made by Hans and Zacharias Janssen in Holland, and even this is in some doubt. The instrument attributed to them consisted of two tubes sliding within a third (see illustration). The longer tube contained the convex object lens and two diaphragms, while the other held a convex eye lens and had a diaphragm at the eye end. The size of the image could be modified by changing the distance between the two lenses, by means of the sliding tubes.

Friends of Galileo have claimed that he, not Janssen, was the inventor of the microscope; at any rate it is certain that as early as 1610 Galileo had made one of his telescopes of much shorter focus than were his first instruments, and had used it to examine minute objects, with great astonishment to himself and friends. He reports the possibility of causing a fly to appear as large as a hen, a magnification of some thirty-five diameters.

Drebbel, a Dutch optician, made similar use of the Keplerian telescope in 1621. It will thus be seen that the microscope has come to us as a development of the telescope, and as the power increased it was found, as in the case of the telescope, a suitable support or stand must be provided in order to secure the requisite steadiness for accurate observation. Even some of Galileo's microscopes, not later than 1840, were mounted on tripods through which they were focused by a screw thread on the body just as are our tripod magnifiers today. The compound eyepiece was invented by Monconys in 1660, a condenser for securing increased illumination by Hooke in 1665, the compound objective by a London optician in 1668, and a binocular microscope by Chevalier D'Orleans in 1685. A fine adjustment screw for focusing was applied by Mr. Joblot in his microscope of 1718. The mirror was first used for illumination by Hertel in 1716. The various instruments of this period were of every conceivable design, large, ungainly, highly ornamented and mostly employed for the diversion of kings and nobles. The barrels were of immense

size, mostly of wood. Many instruments were two feet or more in height. Objects were mounted between bits of glass held in ivory strips, sometimes half a dozen on a strip. We reproduce an old figure of Hertel's microscope to give an idea of the general tendency in the early days of the microscope.

L. B. E.



<p><b>SUBSCRIPTIONS:</b>          One Dollar per Year.          To foreign countries, \$1.25          per Year, in advance.</p> <hr/> <p>Subscribers will be notified when subscription has expired. Unless renewal is promptly received the JOURNAL will be discontinued.</p>	<p>Journal of  <b>Applied Microscopy</b>          and  <b>Laboratory Methods</b></p> <p>Edited by L. B. ELLIOTT.</p>	<p><b>SEPARATES.</b>          One hundred separates of each original paper accepted are furnished the author gratis. Separates are bound in special cover with title. A greater number can be had at cost of printing the extra copies desired.</p>
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We begin our fifth year of publication with the brightest of prospects. Our editorial staff is better organized, and we have as collaborators a list which any periodical might envy. There has never been so much genuine interest manifested in the upbuilding of an American journal of methods, and we now have in hand more original matter than was published during the entire year just completed, in addition to over fifty papers now in preparation which will appear in the present volume. We therefore feel justified in promising a better journal of work for workers than ever before.

The working museum is coming more and more to be a necessity, and the material which in many cases has been simply accumulated as a spectacular accessory to the science department, is now being turned to practical account for study and demonstration. The collection, preparation, arrangement and intelligent use of museum material is a distinct branch of science teaching, in which very few, comparatively, have had much systematic training. The JOURNAL wishes to take up this subject, and invites those having had experience in this work to contribute suggestions and descriptions of methods with photographs illustrating the subject treated of.

The use of the projection lantern for class demonstration is becoming quite general and its value increasingly more apparent. Its successful employment calls for the rapid and accurate use of the photo-micrographic camera, and a practical knowledge of lantern slide printing. The series of papers by Professor Dennis, now appearing, is a beginning in the direction of a fuller knowledge of the many processes and the improved apparatus applicable to this work. The JOURNAL is a "clearing house for methods," and we suggest the passing through of the good ones in these lines, as there are very many needing help in this direction.

Owing to the increased amount of contributed matter it is necessary to reduce the amount of review material somewhat, and to compensate for this decrease the reviews will be held even more strictly to the description of methods than ever before. We have this year subscribed for practically all the foreign periodicals of any consequence, and these will be in the hands of our reviewers at the earliest possible moment with a view to making this service not only as complete as possible but prompt as well.

Our mailing day is now the first day of the month. Subscribers not receiving their copies promptly will please notify us in order that in case the number has been lost another may be sent. Please remember that subscriptions are discontinued immediately upon expiration.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Ferguson, Margaret C. The Development of the Pollen-tube and the Division of the Generative Nucleus in Certain Species of Pines. *Annals of Botany*, 15: 193-223, pls. 12-14, 1901.

The species studied were *Pinus strobus*, *P. austriaca*, *P. rigida*, *P. montana* var. *uncinata*, and *P. resinosa*. Numerous collections extending over a

period of three years yielded a very complete series of stages. The principal results are about as follows:

Pollination, in the vicinity of Ithaca, N. Y., occurs during the last week in May or the first week in June, and fertilization takes place about thirteen months later. The pollen tube begins to grow soon after the pollen grain reaches the nucellus, and the tube nucleus at once passes into the tube. The generative cell—often called antheridial cell—divides, giving rise to the stalk cell and generative cell sometimes before the beginning of winter. The following summer, shortly before fertilization, the nucleus of the body cell divides, but two sperm cells are not formed, the sperm nuclei, on the contrary, remaining surrounded by a common mass of cytoplasm. A difference in the size of these two nuclei early becomes apparent, and in the pollen tube the larger nucleus is in advance. The nuclear reticulum, at first delicate, becomes dense, but there is no indication of a special metaplasmic substance.

In the division of the nucleus of the body cell, the spindle is extranuclear and unipolar in origin and is apparently formed by a transformation of both the cytoplasmic network and the nuclear reticulum. There is thus no definite kinesis in the cell, this division indicating not persistent cell-constituents, but rather, different manifestations of the same thing.

No individualized centrosomes—or blepharoplasts—were found in connection with the formation of the sperm nuclei, but the cytoplasmic radiations which accompany the division suggest that we may have here, still persisting in the cell, the vestiges of such an organ as that described by Webber. c. J. C.

Ferguson, Margaret C. The Development of the Egg and Fertilization in *Pinus Strobus*. *Annals of Botany*, 15: 435-479, pls. 23-25, 1901.

The initial cells of the archegonia can be detected about two weeks before fertilization, which, in *Pinus strobus*, occurs between the tenth and thirtieth of June.

The ventral canal cell is cut off about a week before fertilization. While the writer believes that there is an intimate relation between the egg and the jacket cells surrounding it, she was not able to demonstrate the nature and origin of the proteid vacuoles, but a careful examination of a large number of preparations indicates that there is no passage of nuclei from the jacket cells into the egg, as described by Arnoldi for *Pinus Peuce* (Strobus). No direct evidence of a nuclear origin of the nutritive spheres, such as was described by Ikeno for *Cycas*, could be

obtained. In the development of the egg nucleus, no metaplasmic substance is recognized, but the linin becomes very abundant.

When the pollen tube is ruptured, the sperm nuclei still surrounded by a common mass of cytoplasm, the tube nucleus, the stalk cell, some cytoplasm and some starch grains are discharged into the egg. One of the male nuclei comes into contact with the egg, but before the nuclear membranes of the sex nuclei disappear the chromatin of each nucleus is resolved into a spirem. Each spirem then segments into chromosomes and the two groups remain distinct until the nuclear plate stage. After the first division of this nucleus the two daughter nuclei present a reticular structure in which the maternal and paternal chromatin appear completely fused. At the second division, two chromatic spirems, representing respectively the maternal and paternal chromatin, again appear. There are indications that the third division will be similar. The spindle fibers in the first division seem to arise by a rearrangement of the achromatic nuclear reticula.

The stalk cell disorganizes in the upper part of the egg, and the tube nucleus and the smaller sperm-nucleus may share its fate, but the two latter not infrequently give rise to mitotic figures of more or less definiteness.

Wherever the chromosomes were counted in the gametophyte, the number twelve appeared. Twenty-four chromosomes were counted in the first division following fertilization.

C. J. C.

Andrews, F. M. Karyokinesis in *Magnolia* and *Liriodendron* with Special Reference to the Behavior of the Chromosomes. Bot. Centralblatt. Beihefte, 11: 3-9, pl. 1, 1901.

In the first mitosis in the pollen mother cells the chromosomes are formed as irregular masses without the previous formation of a spirem. The chromosomes are generally U-shaped, though many are in the form of open or closed rings or ellipses. These chromosomes split longitudinally, but in the metaphase and anaphase there is no indication of a second longitudinal splitting preparatory to the second division, and since the daughter nuclei pass into the resting condition the writer believes that no such splitting occurs, and that consequently the identity of the chromosomes from the first to the second division is not maintained. In the second division, the chromosomes arise by the segmentation of an irregular spirem. The number of chromosomes was not determined, but as many as forty-eight were counted in a single polar view.

C. J. C.

Ishikawa, C. Über die chromosomenreduction bei *Larix leptolepis* Gord. Beihefte zum Bot. Centralbl. 11: 6-7, 1901.

Prof. Ishikawa makes the preliminary announcement that in *Larix leptolepis* the mitotic divisions in the pollen mother cells occur as in *Allium fistulosum*, where he has described the first division as an equational—and the second a reduction division according to Weissmann's theory. The paper, with plates, will appear in the Journal of the College of Science.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Troop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be Sent for Review to Agnes M. Claypole,  
N. Marengo Avenue, Pasadena, Cal.

**Atkinson, R. T.** The Early Development of the Circulation in the Suprarenal of the Rabbit. *Anat. Anz.* 19: 23, 24, 1901 (Aug.).

Minot has described the form of circulation found in the embryonic suprarenal and has called it "sinusoidal."

It consists essentially of a system of relatively large spaces of tubes (sinusoids) that communicate very freely with a large vein, the endothelial walls of which are in close apposition to the cells of the parenchyma without the intervention of an appreciable amount of connective tissue. Sinusoids are also found in the liver and pro- and mesonephros. In the liver they form a purely venous system throughout life. In the suprarenal and probably also in the pro- and mesonephros they early become attached to the arterial system. In the adult suprarenal the sinusoidal origin of the circulation is known by the relatively large size of the capillaries and the extremely small amount of connective tissue beneath the endothelium. The condition characteristic of young mammalian embryos persists apparently in the adult of Amphibia.

In 12.5-day rabbit embryos the suprarenal first appears and consists of a few cords of cells lying just cephalad of the mesonephros, closely related to several large sinusoids, the latter communicating freely with the cardinal vein and the sinusoids of the nearby mesonephros. At thirteen days the suprarenal has become much larger, the cells are close together, and the sinusoids more numerous. Their endothelial lining can be seen and they freely communicate with the cardinal vein. At fourteen days the sinusoids are smaller, probably due to the rapid growth of the surrounding parenchyma. An artery comes off from the aorta just above the suprarenal and passes to the opposite side of it, a branch going directly to the sinusoids.

In older rabbits the cells of the parenchyma become compactly massed, the size of the sinusoids is lessened and thus resemble true capillaries, their connections with the vein are reduced in number. Thus it can be seen that the artery becomes early attached to the sinusoids of the suprarenal, the latter rapidly decrease in size and become like capillaries and lose connection with the vein. This method of establishment of the arterio-venous circulation will probably be found to occur also in the mesonephros.

E. J. C.

**Tandler, J.** Mikroskopische Injectionen mit kaltflüssiger gelatin. *Zeit. f. wiss. Mikros. u. f. mikros. Tech.* 18: 22-24, 1901.

The fact that the hardening of gelatin can be delayed by the use of certain salts was utilized in the process of injecting amphibians by adding large quantities of potassium iodide to the gelatin.

The mixture was made in the following way: Five grams of finest gelatin was dis-

solved in 100 c. c. distilled water, warmed slightly. The solution was colored to the desired shade with Berlin blue and then five to six grams of potassium iodide is slowly added. Customarily this mass remains liquid and fit for injection at 17° C. But should it solidify it is only necessary to add more iodide of potassium. A few thymol crystals will make the mixture keep for a month or more if put into a stoppered bottle. A Teichmann's syringe was used, as it had a fine cannula. Whether the animals were warm or cold blooded they were injected immediately after death. The injected objects were then put into five per cent. formalin, which fixed the mass most completely, rendering it absolutely proof to chemicals subsequently applied. Hence it is possible to decalcify the material after hardening the mass in formalin and not in the least soften or otherwise affect the gelatin. Such material may be left for days in hydrochloric or sulphuric acids without injury. The mass has these advantages: It can be kept ready for use for a long time; a gelatin block fixed in formol may remain for days uninjured in acids; the mass penetrates into the smallest vessels and does not diffuse nor fall out of the larger vessels during microscopical sectioning and staining; it is transparent and allows any process of microscopical technique. No change whatever is noticable in the protoplasm. In a section of lung the minutest vessels are injected, the pulmonary artery is filled, and the tissue stained in the usual way. A frontal section of the skull of a triton which has been decalcified, shows the glands with their network of vessels; on each side the nasal wall, the mucous membrane and rich vascularization.

A. M. C.

**Schüffner.** Beiträge zur Kenntniss der Malaria. Deutsches Arch. für klin. Med. 69: 428-449, 1. tafl., 1899.

The author considers the study of fresh malarial blood of little use owing to the extremely small size of the parasite of typical malaria. Complete fixation is secured by long continued drying in the air. After two to three days the water still shows traces of hemoglobin, and after two to three weeks almost none goes into solution. The film is hardened, the albumen has lost its power to swell, and the hemoglobin its solubility. Simply drying does not give this air-fixation, as hemoglobin may stay a month unchanged in the drying oven. The change takes place more rapidly in damp air than dry, on damp days than dry, and hence must be a chemical change. Such blood films can be hardened too long and then do not take the usual stains. This can be prevented by drying over sulphurous acid or calcium chloride. It is well after air-fixation for six to thirty-six hours, in the climate of Sumatra, to treat the preparations with Mannaberg's picric-acid solution or alcohol or sublimate. The author also uses a one per cent. solution of formalin with five to ten per cent. glycerin. Many preparations are lost by drying from the skin (ammonia?) of the patients. After the air-drying is complete the preparations should be protected from moisture and strong sunlight. The whole procedure is as follows: 1. Draw out the blood drop onto a slide. 2. Air-dry in a place somewhat shielded from sunlight for six to thirty hours. 3. Lay carefully in a dish of five per cent. glycerin and formalin, film downwards, keeping the film from contact with the bottom, for five or ten minutes. 4. Lay in spring water for fifteen to sixty seconds. 5. Stain in hematoxylin, according to the strength, for one to ten minutes. 6. Wash out. 7. Dry, mount in Canada balsam. 8. Examine first with low powers. In preparations so treated the background is formed of a delicate blue layer of red blood cells, from which the remaining elements of the blood are sharply differentiated. The form-preservation is much better than by Mannaberg's method.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Rhumbler, L. Nordische Plankton-Foraminiferen. No. XIV aus Nordisches Plankton herausgegeben von Prof. Dr. Karl Brandt. 32 pp., 33 fig. im Texte. Lipsius und Tischer. Kiel, 1901.

This is one of a series of papers on the various organisms of the plankton of the North Atlantic Ocean by specialists, the whole to form a volume when

completed. It is the intention to include all forms, both plant and animal, known to occur in the plankton north of 50° north latitude. The *Foraminifera* of the plankton as a rule possess many chambered calcareous shells wound in a spiral which in *Orbulina* is covered by an outer surrounding chamber. The pelagic species thus belong to the *Rotalida* with the single exception of *Chilostomella*, an elongated form devoid of spiral structure. As elsewhere within the group no distinction between ectoplasm and endoplasm can be detected. Many of the pelagic species contain an orange or yellowish pigment which seems to depend upon the food of the individual. The genus *Pulvinulina* feeds upon diatoms and radiolarians, while *Globigerina* apparently confines itself to the *Copepoda*, for the partially digested muscle fibres of these animals are to be found in the protoplasm of members of this genus. The absence of the skeletal parts of the *Copepoda* in the protoplasm is explained by the extrathalamous capture and partial digestion of the prey. The protoplasm of certain pelagic species contains a peculiar network of threads resembling a mycelium, though apparently not of parasitic origin. These problematical structures stain a bright blue in methyl green-eosin and are apparently of a gelatinous composition. The suggestions are made that they may serve as supports for pseudopodia, or that they may be (in *Globigerina*) the early stages in the formation of a gelatinous covering about the shell. The genera *Globigerina* and *Orbulina* harbor the commensal alga, *Zooxanthella*.

The nucleus of all the species examined (by the sectioning method) was single, and in *Globigerina* fixed in sublimate exhibits a peculiar alveolar structure without chromatin network, while in *Pulvinulina* the typical nuclear structure is to be found. The absence of developmental stages in the plankton material leads the author to suggest that reproduction takes place in the bottom waters. The collections examined contain some hints of an alternation of generation in the pelagic types, of embryos within the maternal organism which become megaspheres, and of swarming individuals which become microspheres, the latter, however, developing into the larger type of adult. This may account for some of the variability in the groups which the author recognizes in the large number of synonyms which he lists. Adaptations to the pelagic life are to be found in the spinous prolongations of the shells which reach two to three times the diameter of the shell body; also in the gelatinous coverings of some species. These structures increase the floating capacity of the organism.

For the study of shell structure material killed in osmic acid is to be preferred since it preserves the organic constituents of shell wall and spines. The protoplasm is well preserved, as are also the symbiotic algæ, but nuclei suffer. Picro-sulphuric renders identification impossible since it destroys the shell, but the preservation is good. Iodin alcohol and sublimate preserve both shell and contents well, but not the *Zooxanthellæ*. Best results were obtained with Schaudinn's mixture ( $\frac{2}{3}$  sat. aq. sol. sublimate +  $\frac{1}{3}$  abs. alc.) heated to 40° Cels. Differential staining was accomplished with the author's methyl green-eosin mixture :

70 per cent. alcohol	-	-	-	300 c. c.
Methyl green	-	-	-	1 gram.
Eosin	-	-	-	1 gram.

Dilute each 100 c. c. with 60 c. c. water before using. Stain twenty-four hours. The decalcified shell stains violet, protoplasm and nucleus both bright red but well differentiated, gelatinous fibers bright blue, and the by-products of digestion, bluish red to green. Decalcification is best done in slightly acidulated alcohol or in Müller's fluid.

C. A. K.

Jennings, H. S. Synopses of North American Invertebrates, XVII. The Rotatoria. Am. Nat. 35: 725-777, with 171 figures, 1901.

This is one of the most important numbers in the series of synopses of North American invertebrates appearing in

this journal. Two hundred and forty species are included in the key, and fully two-thirds of these are figured. The paper is critical and authoritative, and will be useful to all who wish to work with this widely distributed and most interesting group of organisms.

C. A. K.

Carpenter, W. B. The Microscope and its Revelations. Eighth edition, enlarged and revised by W. H. Dallinger. 1181 pp. 23 pl. 1901. P. Blakiston's Son & Co., Philadelphia. \$8.00.

The chapters dealing with the microscope, accessory apparatus, microscopical methods, and the application of the microscope to geological investigation,

constituting about one-half the book, have been entirely rewritten and enlarged. The discussion of the mechanical and optical parts of the microscope is quite up to date, and others than English makers receive ample and courteous treatment, the work of American firms winning many words of commendation. The editor has adopted a classification of microscopes which may prove of value to prospective purchasers of microscopes. The chapter upon methods is condensed from Lee's "*Vade Mecum*" and is good as far as it goes, though the omission of formalin and the Golgi method is to be deplored. The subjects of bacteriology, botany, and zoölogy are handled by eminent specialists, but have not been rewritten in the present edition. Certain sections, as for example, that on the nervous system, should receive attention in the next edition. Zoölogical subjects, as heretofore, predominate over the others in the chapters devoted to the revelations of the microscope. All who use the microscope will, however, find this volume a most useful one, for it presents very fully the theory and principles involved in the construction of the instrument in their latest developments and applications. For the amateur it furnishes a valuable compendium of applied microscopy.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Onuf (Onufrowicz), B., and Collins, J.** Experimental Researches on the Central Localization of the Sympathetic with a critical Review of its Anatomy and Physiology. *Arch. of Neurol. and Psychopathol.* 3: xi. and 252, pl. 1-9, 1901.

logical and morphological standpoints. Nearly a half of the work is devoted to this discussion of earlier literature, and this portion alone, leaving out of account the authors' positive contributions, will make it very valuable as a reference handbook. Fifteen chapters are devoted to this digest of the literature, with the following grouping: "Part I, The Anatomy of the Sympathetic System," including in five chapters a very clear and fairly detailed account of the gross anatomy, histology, and embryology of the different parts of the sympathetic system. Text diagrams make plain the different views as to the courses of the fiber tracts. Part II discusses in the same way the "Physiology of the Sympathetic Nervous System," including chapters on "Secretory Functions," "Vascular Functions," "Cardiac Functions," "Respiratory Functions," "Influence upon Involuntary Automatic Movements," "Trophic and Tonic Functions," "Reflex Action of Sympathetic Ganglia," and "The Functional Interrelation of the Sympathetic and Cerebro-Spinal System."

The authors' original experiments were made on young cats and had as their basis the study, by means of the Marchi, Nissl, and Pal methods, the degeneration tracts in the central nervous system (spinal cord and medulla oblongata) after extirpation of different parts of the sympathetic system. Their principal conclusions as to the relation of the fibers of the sympathetic system to the central nervous system may be summarized as follows: the afferent (sensory) fibers of the sympathetic nerves have their cells of origin in the ganglia and plexuses of the sympathetic system itself and not, as claimed by Kölliker, in the spinal ganglia. These different fibers are connected by their terminal arborizations with the cells of Clarke's column in the spinal cord. The efferent fibers take their origin in the following cell groups in the cord: 1st, the paracentral group; 2d, the small cells of the lateral horn; 3d, probably also the cells of the intermediate zone. In the oblongata the vago-glossopharyngeal nucleus gives rise to the visceral efferent fibers of the vagus-glossopharyngeal, while the somatic efferent fibers, i. e., fibers to the striated muscles, arise from the nucleus ambiguus.

The physiological results bring out very clearly the importance in this kind of work of studying the compensatory or regulatory functions which may develop a considerable time after the removal of parts of the nervous system. It was found in some cases that functions, e. g., sweat secretion, pupil dilation, which disappeared immediately on extirpation of the stellate ganglion, would after some

As is indicated by the title, this monograph contains a very complete summary and critical review of the work which has been done on the sympathetic nervous system, both from the physio-

weeks reappear in practically normal completeness, apparently then being subserved by some nervous mechanism which under normal circumstances contributed very little to their performance.

A final section is devoted to the "Pathology of the Sympathetic." The plates illustrating the paper are excellently done. R. P.

Aschkinass, E., and Caspari, W. Ueber den Einfluss dissociirender Strahlen auf organisierte Substanzen, insbesondere über die bakterienschädigende Wirkung der Becquerel-Strahlen. Arch. f. d. ges. Physiol. 86: 603-618, 1901.

The purpose of this investigation was to determine the physiological action of radiations of short wave lengths, including Röntgen, kathode, and Becquerel rays.

The first method used was the study of the amount of gaseous metabolism in frog muscle in tubes exposed to Röntgen and Becquerel rays as compared with that in muscle similarly treated but not exposed to the action of the rays. The results from these experiments showed that these rays caused a lowering of the vital activity of the muscle as indicated by the amount of the metabolism. The experiments were not performed in sufficient number to be absolutely conclusive, however, and attention was turned to the effect of Becquerel rays on the growth of *Micrococcus prodigiosus*, cultures on nutrient agar. The preparation of a typical experiment was as follows: the under surface of the cover of a Petri dish was coated with agar and the center of this was inoculated with *M. prodigiosus*. Directly below the point of inoculation on the bottom of the Petri dish was placed the brass capsule containing the radio-active substance (1g. of barium-radium-bromide crystals). The culture was exposed from one to five hours to the action of the rays. It was found that the radiations of the sort that are only slightly absorbed in their passage through any medium were without effect on the growth of the bacteria cultures. Growth proceeded as well as in the controls. On the other hand, the rays which are absorbed in high degree caused a complete inhibition of the growth of the colonies. The experiments were varied in such ways as to prove that the effect is due only to the action of the Becquerel rays on the bacteria themselves. The authors hope to carry on similar experiments with cultures of pathogenic bacteria with a view of possibly finding some therapeutic value in the use of Becquerel rays. R. P.

Viguer, C. Fécondation chimique ou parthénogénèse? Ann. Sci. natur. Zool. Ser. XII, T. 9: 87-138, 1901.

Viguer finds that unfertilized eggs of the echinoderms *Sphærechinus granularis*, *Toxopneustes lividus* and *Arbacia*

*pustulosa* taken at Algiers will develop parthenogenetically in normal sea water, or are, as he puts it, "naturally parthenogenetic." The  $MgCl_2$  solutions which Loeb found to be effective in producing artificial parthenogenesis in the eggs of starfish and sea urchins taken in this country, have, according to Viguer, a retarding effect on the development of the eggs of the Algerian species, and may even inhibit entirely the "natural parthenogenesis" which would under ordinary circumstances occur. The plutei from parthenogenetically developed eggs of *Arbacia* are found to be different from those arising from normally fertilized eggs in the lengths of the arms and the angles included between them. The author very strongly condemns the use of the eggs of these species which will naturally develop parthenogenetically, for the study of artificial parthenogenesis. R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Flexner, S.** The Pathology of Bubonic Plague. Univ. of Penn. Med. Bulletin, 14: 205, 1901.

Flexner gives the results of his study of the lesions of bubonic plague as shown in material gathered in Hong

Kong and in San Francisco. To this he adds the results of histological study of experimental plague in guinea pigs and a historical review of our knowledge of the disease as a whole. The article may be regarded as in part a confirmation of the existence of true plague in San Francisco, and in part as a contribution to knowledge of the lesions produced by the disease in man and animals. He recognizes the fact that two types of the disease exist, the bubonic and the pulmonic, and points out that the former was the only form found in the San Francisco outbreak. The histological changes in man are treated at length, and attention is drawn to the similarity of certain cell changes with those in other diseases. He emphasizes the similarity between the lesions in man and those experimentally produced in animals; viz., primary bubo, necroses in liver and spleen, minute cell changes, and proliferative nodules. All but the latter he considers identical in man and animals. He suggests that further study will show that the proliferative nodules in animals also have a counterpart in the human lesions.

W. R. BRINCKERHOFF.

**Herxheimer, G.** Ueber supravasale Pericard-Knötchen und Sehnenfleck. Virch. Archiv. für path. Anat. 165: 248, 1901.

Herxheimer studies histologically the nodules distributed along the epicardial surface of the coronary arteries in five

cases occurring in twenty-three consecutive autopsies. These are generally limited to the ventricles, and occur most frequently in the region of the bifurcation of the vessel. In only one case does the vein show any. In each case milk patches occur. The nodules bear no relation to the adventitia of the vessel, but develop from the connective tissue lying between the endothelial and elastic layers of the epicardium.

They consist of dense connective tissue arranged in lamellæ parallel to the surface. The periphery is the most cellular portion. In some nodules there are spaces lined by cubical endothelium. The nodules begin as a localized thickening of the epicardium over an artery. Changes in the artery itself are infrequent and inconstant. Herxheimer finds no constant change in the external elastic lamella such as Knox (Jour. of Exp. Med., 4: 245) describes and regards as the primary change.

The structure and origin of these nodules, according to Herxheimer, agrees with that described by Meyer and Ribbert, for milk patches, except that, in the latter, spaces lined by endothelium are more frequent. Finally in his cases he describes transitions between supravascular nodules and milk patches.

In conclusion he regards the formation of the nodules and milk patches as identical pathological processes, and considers their etiology as referable to mechanical causes from high and irregular blood pressure producing chronic irritation of the epicardial surface, especially on exposed parts. For neither does he support the theory of an origin from a localized pericarditis nor from developmental irregularities in the epicardium.

H. A. CHRISTIAN.

Murawieff, W. Die feineren Veränderungen durchschnittener Nervenfasern im peripheren Abschnitt. *Ziegler's Beiträge zur path. Anat.* 29: 103, 1901.

The author found the "formal-methyl" method of great service in the study of both normal and pathological nerve fibers. It was devised several years

ago by Rossolino and Murawieff.

The fresh nerve is placed in a four per cent. formalin solution, where it should remain not less than two or three days. It is then transferred to 95 per cent. alcohol for twenty-four to forty-eight hours, but never longer than forty-eight hours. Tease out a small piece of the nerve, thus hardened, and stain for ten to fifteen minutes in a one-half per cent. aqueous solution of methylen blue which is heated until the first bubbles appear. Cool the solution and move the tissue into anilin oil for one-half a second (anilin oil one part, 95 per cent. alcohol nine parts), then into 95 per cent. alcohol for one-half a second. Clear in cajeput oil. Place the bits of nerve on a slide, add a drop of Canada balsam. Tease the nerve fibers thoroughly and cover the preparation with a cover-slip.

If one wishes to make a cross section of the nerve, after hardening in four per cent. formalin, place it in 95 per cent. alcohol twenty-four hours, then in absolute alcohol twenty-four hours. Embed in celloidin. Stain in the manner already described.

J. H. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

*Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.*

Chester. *A Manual of Determinative Bacteriology.* Macmillan & Co., p. 400. Price \$2.60.

Prof. Chester, of the Delaware College Agricultural Experiment Station, has

done great service to bacteriologists in the publication of this work. He attempts to give a brief determinative description of all species of bacteria which have been sufficiently described for the purpose. Two preliminary chapters briefly describe methods of characterizing species. In these chapters he eliminates long descriptive phrases and puts in their places short characteristic terms, which very greatly reduce the number of words. The bulk of the work is a compilation of bacteria descriptions. The groups are divided according to Migula's recent classification and, by the use of analytical keys and family synopses, it is made easy for one to determine rapidly where a given bacterium should be classified. The work does not pretend to contain a full, systematic description of

bacteria, but is designed, rather, as an aid in determining their classification. In the work, Chester has given names to all unnamed species of bacteria described. If the person who originally described the organism gave it simply a number, Chester has given it a name, so that all organisms in this work go by distinct names. Naturally, therefore, some of the organisms would not be recognized by their authors under the names given them by Chester. In all, 800 different species of bacteria are described. The immense amount of labor involved, in searching through the literature and in properly arranging the bacteria so variously described, may easily be conceived.

Of course, in a work of this sort, specialists who have studied certain small groups of bacteria will inevitably find omissions, and points to criticise; but the work, nevertheless, is very satisfactory and useful. Indeed, this book will, hereafter, be indispensable to a bacteriologist's laboratory. Bacteriologists of America and, indeed, of the world, owe a debt to Prof. Chester for the labor involved in this publication.

H. W. C.

**Fischer, Alfred.** The Structure and Functions of Bacteria. The Clarendon Press, Oxford. Translated by A. Coppen Jones.

Prof. Fischer's work upon bacteriology has been so well known in the original that it is hardly necessary to do more

than refer to this excellent and useful translation. No other book, among the numerous treatises on bacteriology, exactly fills the place of this one. The various aspects of present day bacteriological problems are very critically and excellently treated. Prof. Fischer's leading position among European bacteriologists is enough to guarantee the value of the work. In some places he has clearly been led by his own views to take a position contrary to the present attitude of bacteriologists. For example, he takes the position that bacterial diseases of plants are an impossibility and do not exist, a position which, since the publication of the original edition, has been vigorously combated and refuted by E. F. Smith of the Agricultural Department. But in spite of such slips as these, the work is one of the very best of the treatises on bacteriology. Translated into English, it is in a much more usable form for English speaking investigators, and the edition which is issued by the Clarendon Press cannot fail to find its way upon the shelves of most investigators, nor to be widely used.

H. W. C.

**Revue Generale du Lait.** Vol. 1, No. 1. Edited by L. Gedoeist, 31 rue Jourdan, Brussels.

Under the above title has appeared in October the first number of a new publication to be devoted to the scientific study of milk.

The editor has associated with himself a considerable number of scientists from various countries of Europe and America. The design of the publication is to review the scientific study of milk from practical, legislative and scientific standpoints. It devotes considerable attention to bacteriological and chemical study. It is to contain original articles and also careful synopses of publications which appear in various countries. It is the only publication devoted wholly to the scientific study of milk. The importance of milk as an article of commerce and scientific interest has justified the appearance of such a journal. The editor and publisher ask for assistance in the way of articles and subscriptions from all interested in the subject.

H. W. C.

**Ramsay.** The Scientific Roll of Bacteria. R. L. Sharland, Churchfield Road, Acton, London, W. An extremely useful publication for bacteriologists is this Scientific Roll of bacteriological literature. It aims to be a complete list of bacteriological papers, the title and place of publication being given in all cases. The three numbers now issued comprise numerous publications, from the early ones of Leuwenhoek, in 1680, to the end of the year 1901. The Roll is a regular publication and invaluable to bacteriologists. The compiler invites the aid of all bacteriologists in making the list complete, by sending him titles of all bacteriological papers, and reprints where possible. His address is, Alexander Ramsay, 4 Cowper Road, Acton, London, W.

H. W. C.

**Widal and Ravaut.** Cytodiagnosis. International Congress at Paris, 1900. *Cytodiagnosis* consists in determining the nature of a pathogenic liquid by the study of the cells it contains. Widal and Ravaut have given the results of their researches at the Soci  t   de Biologie and the Soci  t   Medicale des Hospitaux. The following is their method:

A few centimeters of liquid is sufficient for the examination. If the liquid shows no coagulum it can be examined direct, but if coagulated it must be shaken with glass beads, and, if very turbid, it is decanted to avoid the fibrin, and then centrifuged.

The examination can be made without staining, and it is easy to count the corpuscles, and even the leucocytes between the desquamated epithelial cells. For this purpose a hematometer may be used, care being taken not to dilute the material too much. It is useful to count the number of both red and white corpuscles in a cubic millimeter. It is necessary also to study a stained specimen. A drop of the residue from the centrifuge is placed upon a slide with a pipette, and stained with thionine, eosin, hemateine or triacide.\* The material is fixed, preferably by alcoholic-ether if they are to be stained with thionine, eosin or hemateine, but in toluene at 110  C. if they are to be colored in triacide. The preparations may also be dried in a drying oven for a proper length of time, and then fixed with absolute alcohol.

Pathogenic liquids exuded from the pleural or abdominal cavities, from the vagina and testis, from the joints and the subarachnoid space, have been examined by different observers by the method of Widal and Ravaut. A large number of important results have been obtained from the work.

Sero fibrous streptococcic pleurisy is characterized almost exclusively by the presence of polynuclear leucocytes; pneumococcic pleurisy by red corpuscles, and some lymphocytes, but always by polynuclear leucocytes, and a greater or smaller number of mononuclear leucocytes, of which some are true phagocytes which engulf the polynuclear cells in their protoplasm.

Pleurisy "a frigore," which is commonly of tuberculous nature, produces a discharge of lymphocytes. Mechanical pleurisy of the heart shows liquids filled with red corpuscles and few lymphocytes. Its chief characteristic is the presence of large cells, like the endothelial cells which are separated from the pleural surfaces.

The study of hydrocele liquids and those from the chord and brain have given results of great interest. Chronic diseases of the nervous system can be studied by this method with equal advantage.

From these examples it may be seen that the method is both novel and useful.

A. GIRAULD, Translated by H. W. C.

\*I do not know these stains and give the French word.

## NEWS AND NOTES.

**A NEW AND RAPID METHOD OF STAINING THE CHROMATIN OF THE MALARIA PARASITE; A NEW BLOOD-STAIN.**—Dr. L. B. Goldhorn, in the New York University Bulletin of the Medical Sciences (Vol. 1, No. 2), gives in detail a method which he has worked out for staining blood with his well known polychrome methylen blue mixture. He claims for the method that it shows, in addition to all that may be seen with other stains, a cell-degeneration in infected blood corpuscles which has not been seen heretofore. The method pursued is as follows :

1. Fix *fresh* smears, after drying, in pure methyl alcohol or in "synthol" for fifteen seconds.
2. Wash in running water.
3. Stain in 0.1—0.2 per cent. aqueous solution of eosin for seven to thirty seconds.
4. Wash in running water.
5. Stain in solution of polychrome methylen blue for thirty seconds to two minutes.
6. Wash *thoroughly*.
7. Dry by rapid agitation in air; not by use of filter paper or by heat.

Rapid fixation by short immersion in methyl alcohol was found as satisfactory as the longer methods.

No special brand of eosin is needed, and if desired erythrosin may be used instead of eosin.

The polychrome solution is prepared as follows: "Two grams of methylen blue are dissolved in 300 c. c. of warm water and four grams of lithium carbonate are added under constant shaking. Pour the mixture into an open porcelain dish over a water bath, allowing the boiling water to touch the bottom of the dish. Stir frequently with glass rod. Remove after fifteen to twenty minutes and pour into a glass-stoppered bottle without filtering; a bit of cotton may, without disadvantage, be used in the funnel. Set aside for several days; then correct the reaction of the solution by cautious addition of four or five per cent. acetic acid solution, until the dye is only very faintly alkaline. This point is not very readily found; it is probably best to acidify the dye first until blue litmus paper shows faint pink above the blue portion where the stain has dyed the paper, and then work backwards, until the stain gives the desired reaction. Trial blood smears should be used to check the result. When the dye is of the proper reaction it takes a good deal of eosin out of the red cells without giving them a blackish appearance. A faintly acid dye gives fine chromatin staining, but degenerative changes in the red blood corpuscles are not brought out. Similar results are obtained by staining too long in the eosin, or by staining in a strong eosin solution."

Certain difficult forms of malarial parasites give excellent preparations with the polychrome stain. With ten seconds immersion, the nucleus of the "ring

form" stains readily. The nuclei of the segmenting bodies are also readily shown, but to demonstrate the loosened-up chromatin of intermediate forms requires immersion of one and one-half to two minutes. To demonstrate chromatin in crescents, staining for one to two minutes is desirable. The usefulness of the polychrome methylen blue method is not restricted to malarial parasites; but has been tried successfully in pernicious anemia, necrosis and polychromatophilia. Nuclei and intra-nuclear network of nucleated red cells; granules of neutrophilic and eosinophilic leucocytes; nucleoli and basophilic granules of lymphocytes, blood platelets and other difficult structures are shown. C. W. J.

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CLEANING SLIDES AND COVER-GLASSES.—Until one has had some experience in microscopical work, the matter of cleaning slides and covers for mounting objects is thought to need little or no attention; but it must be remembered that all of the light that enters the microscope, except when viewing opaque objects, must pass through these plates of glass. Except for critical microscopical work no cleaning agents or special mixtures are necessary. New slides may be cleaned sufficiently for ordinary work by thoroughly rinsing them in clean water or, better, by soaking them a short time in alcohol and, after allowing them to drain on a clean cloth or blotting paper, wipe them with a soft cloth, such as cheese-cloth, or with filter paper. In wiping, as in all handling of clean slides, they should be held by their edges, since the slightest touch on the surface leaves a finger mark. Slides upon which objects have been mounted in glycerin, balsam, or other gummy media, may be cleaned by allowing them to soak for some time in alcohol, benzine, or turpentine, followed by thorough washing with soap and water. A good method to follow with balsam is to heat the slide until the balsam melts and remove the cover, after which the balsam is more easily removed, as indicated above. Cover-glasses may be cleaned by the same procedure as given for slides. More care must be exercised to avoid breakage, and the covers should be put into the cleaning agents one by one, else they stick together. In wiping covers hold the cover by its edges between the thumb and forefinger of one hand, and with the thumb and forefinger of the other hand, covered with a soft, clean cloth or, better, with lens paper, rub the surfaces. Breakage will be rendered less liable if the thumb and finger are kept exactly opposite each other. One may determine whether or not the cover is perfectly clean by looking through it towards the light. Particles of dust may be removed by breathing on the cover and wiping again carefully. Clean covers should always be handled by their edges or with fine forceps.

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Prof. C. C. Nutting of the University of Iowa will direct the work on marine invertebrates during the coming cruise of the U. S. Fish commission steamer Albatross to the Hawaiian Islands.

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Mr. Thomas Meehan, editor of *Meehan's Monthly*, and well known for his work in horticulture and botany, died on November 19th. During his life of seventy-five years he did much to advance the knowledge of the subjects to which he devoted his attention and was a valuable member of many scientific societies.



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### **Modification of Eosin and Methylen Blue Contrast-staining, with Technique.**

Eosin and methylen blue contrast-stains have long been recognized as extremely valuable in histological and pathological work, but many workers have been deterred from their use by the difficulty of the technique, the lack of uniformity in the results, and the comparative instability of the stain.

The best method of using these stains at the present time is that employed by Councilman and Mallory, but however well the stain has been perfected, there have been many difficulties. The slight modifications employed by the author consist in the addition of more potassium carbonate, making a stronger alkaline methylen blue solution, and using a weaker solution of eosin. These modifications together with the definite instructions regarding technique enable one to carry through a large number of sections in a comparatively short time with uniformly more excellent results than have hitherto been obtained. The method also seems to increase the permanency of the stains.

The entire technique, including the fixing, hardening, imbedding, and staining of tissues, is given in detail :

1. Harden pieces of tissue from 3 to 5 mm. in thickness in Zenker's fluid <sup>1</sup> for twenty-four hours.
2. Wash twenty-four hours in running water.
3. Trim specimen to maximum size of 5 x 15 x 15 mm. and dehydrate in 95 per cent. alcohol for twenty-four hours.
4. Absolute alcohol twenty-four hours.
5. Chloroform twenty-four hours.
6. Chloroform and paraffin twenty-four hours.
7. Paraffin at 120° F. four hours.
8. Imbed and section specimen. Sections of internal organs should be cut 8 to 10  $\mu$ ; central nervous tissue, 5 to 8  $\mu$ .

1. Zenker's fluid is the only satisfactory hardening and fixing reagent for this method, although corrosive sublimate, Mueller's fluid, or Flemming's solution can be used.

9. Allow sections to flatten by placing in water at a temperature of 110° F.

10. Fix on slide with Mayer's albumen fixative, which should not be over one month old. Remove section from the water by inserting slide with a thin film of the fixative beneath specimen, and lifting gently. Remove excess of water with a dry cloth, care being taken not to touch section.

Insert slides in a tray<sup>2</sup> and place in the incubator at 98° F. for one to three hours; they may stand in sunlight the same length of time, or in a warm room two to six hours. (Shrinkage occurs if sections are allowed to dry, especially brain sections, for which one hour is sufficient.)

11. (a) Dissolve paraffin from sections by filling tray with xylol, which is allowed to remain from one to two minutes.

(b) Remove xylol and wash with 95 per cent. alcohol for one minute.

(c) Remove alcohol and immerse sections with tincture of iodine, which is left from three to five minutes.<sup>3</sup>

(d) Remove iodine and wash thoroughly in running water two minutes. (Water is allowed to run into tray gently.)

(e) Remove excess of iodine from section by covering slides with 95 per cent. alcohol for one to two minutes.

(f) Remove alcohol and fill tray with 4 per cent. aqueous eosin (aq. yel.)<sup>4</sup> allowing it to stand for three-quarters to one hour.

(g) Remove eosin solution and wash in running water for half a minute, to remove excess of stain.

(h) Fill tray with the methylene blue solution :

Methylene blue	-	-	-	-	1 gram.
Potassium carbonate	-	-	-	-	1.5 grams.
Distilled water	-	-	-	-	100 c. c.

Dilute one to ten at time of using. Stain for one and one-half to two hours, but never over.

12. Decolorize and differentiate with 95 per cent. alcohol. For this purpose, four to six Petri dishes are partially filled with alcohol; slides are removed from tray, excess of stain wiped off, and slide placed face up in Petri dish (two slides in each dish). This process usually requires about five minutes, but not over ten, and the section must be examined from time to time under the microscope, care being taken, however, not to allow sections to dry. When the differentiation is complete, the nuclei are a brilliant blue, while the protoplasm of the cells, blood vessels, muscle fibers, etc., are a dull red. Chromatin particles of nuclei should stand out distinctly in well stained sections.

13. Transfer to absolute alcohol contained in oblong glass tray, large enough for one specimen (tray being kept covered), for one or two minutes.

Transfer to xylol after removing excess of alcohol. Clearing should take place immediately if sections are dehydrated sufficiently. When economy must

2. This tray is made of copper, with corrugated sides, and may hold from six to twenty-four slides. In laboratories where specimens from an entire autopsy are carried through, trays containing twenty-four or more slides are desirable.

3. This is to remove excess of corrosive sublimate when used in the fixing reagent.

4. This and the above solutions may be used again and again, providing the strength is not allowed to deteriorate.

be practiced, clearing may be brought about by blotting sections on fine blotting paper during the transfer from differentiating alcohol to "absolute" alcohol, or from absolute alcohol to xylol, care being taken not to let section dry. Mount (from xylol) in xylol balsam.

Alcohol in which sections are decolorized and differentiated is discarded when the stain no longer seems to mix with it, but clouds the fluid. Alcohol and xylol for clearing work better when fresh. When the methylen blue solution is not sufficiently alkaline, or when the eosin solution is not of sufficient strength, purple stained sections with little differentiation will be produced; too much emphasis therefore cannot be laid upon the necessity of following the details of the process.

This method possesses the following values :

1st. Many sections may be stained at the same time (one hour and a half being a sufficient length of time for decolorizing, clearing, and mounting twenty-four sections).

2d. Bacteria are stained if present.

3d. Uniformly stained sections are obtained.

The only objection to this, as to all anilin stains, is that the sections lose their brilliancy after five or six years. Sunlight fades them rapidly.

Laboratory of Connecticut Hospital for Insane.

ROLLIN H. BURR.

## LABORATORY OUTLINES.

For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### INTRODUCTORY SUGGESTIONS.

The following outlines are designed for those who have access to little apparatus outside of a good microscope, like the BB4. Although the course will not cover as much ground as the author believes should be given to such work, it will probably be more than sufficient for the time usually allotted to such courses in most of our colleges.

Whatever may be the individual opinion in regard to the first course in botany, it is the writer's belief that the second course should be largely carried on with the use of the compound microscope; and should cover, in a general way, the whole plant kingdom, so that the idea of the evolution of plants and their natural relationships will be made prominent. The student should have a general grasp of the plant kingdom as a whole, and to accomplish such a result a large number of forms must be studied. Along with this general idea, a considerable knowledge of morphology and physiology may be acquired, since the study should have to do largely with living material. After such a course the student is well fitted to take up the various departments of advanced work. He will have acquired a sufficient knowledge of biology to carry on intelligently whatever special studies he may later choose to pursue, as anatomy, histology, cytology, physiology, ecology, taxonomy, or advanced work in special groups.

It is often supposed that to accomplish good work it is necessary to have on hand an expensive equipment and all the facilities which our leading colleges afford. There is, however, plenty of original work that may be done by those who do not have such an equipment, and substantial progress may be made in the general facts of the science with little besides what is indicated below.

This course will suppose an elementary knowledge of botany equivalent to a half-year course in any good high school. The student should have the following equipment:

1. A text-book for general reading, such as *PLANT STRUCTURES (A Second Book of Botany)* by John M. Coulter, 1900.
2. A compound microscope, like the BB4, having a double nose-piece with  $\frac{2}{3}$ -inch and  $\frac{1}{4}$ -inch objectives, and 2-inch and  $\frac{3}{4}$ -inch eyepieces. The BB8 stand with complete substage and triple nose-piece is preferable if one can afford to pay the difference in price.
3. A number of slides and cover-glasses.
4. A good hand lens or a dissecting microscope.
5. A good note-book with note paper and smooth drawing paper, and also some bristol board drawing paper for extra fine drawings. (See "The Laboratory Note Book," *JOURNAL OF APPLIED MICROSCOPY* 3: 887-888.)
6. Loose writing paper for making temporary records and calculations.
7. Two good lead pencils, a No. 3H and a No. 6H. It is also desirable to have a bottle of India ink and a crow-quill drawing pen so that some of the drawings may be finished in India ink.
8. The following instruments are necessary:
  - a. A pair of forceps.
  - b. Several medicine droppers.
  - c. Some needles set in wooden or bone handles.
  - d. A scalpel.
  - e. A razor.
  - f. Dishes, watch glasses, and bottles of various sizes.
  - g. Plenty of clean cotton rags and some paper blotters.
9. The following simple reagents will be needed:
  - a. A small bottle of 50 per cent. aqueous solution of glycerin.
  - b. A bottle of distilled or pure, boiled water.
  - c. Iodin solution.

Make a strong solution of potassium iodide in distilled water; to this add crystals of iodine until a saturated solution is obtained. This may be diluted with distilled water until it is of a clear reddish-brown color.
  - d. Salt solution, five or ten per cent. aqueous.
  - e. A bottle of ninety-five per cent. alcohol.
  - f. Ripart and Petit's solution.
  - g. Carbolic acid.

If a greenhouse is not near, a window garden and aquarium become indispensable. Water plants kept in glass jars with some small water animals, as

water snails and water beetles, will usually grow with little or no attention. In most cases the jars should be covered.

Many of the specimens may be preserved in various preserving fluids, and some may be dried. These will be found very convenient in case fresh material cannot be obtained when desired.

Microscopic plants may be preserved in water, in homeopathic vials, provided a drop of carbolic acid is added to each bottle of material.

Plants like mosses, liverworts, fleshy fungi, stems, roots, rhizomes, etc., may be preserved in 70 to 80 per cent. alcohol.

The ordinary filamentous algæ are usually well preserved in Ripart and Petit's solution.

Myxomycetes in the fruiting stage, woody fungi, lichens, some liverworts, and many other plants may be kept in a dry condition in ordinary paper boxes.

Ripart and Petit's solution :

Camphor water	-	-	-	-	-	75 c. c.
Distilled water	-	-	-	-	-	75 c. c.
Crystalized acetic acid	-	-	-	-	-	1 gram.
Copper acetate	-	-	-	-	-	0.3 gram.
Copper chloride	-	-	-	-	-	0.3 gram.

To make the camphor water, dissolve four grams of camphor in eight c. c. of alcohol. Distribute this on clean cotton and expose until nearly all of the alcohol is evaporated; then place the cotton in a funnel and pour distilled water through it until 500 c. c. of percolate are obtained.

For preserving algæ, take a two per cent. solution of formalin and add to it from three to five per cent. of the above solution.

General pharmaceutical rule for making any lower grade or percentage of alcohol from any given grade or percentage.

Take of the grade at hand as many volumes as the number of the per cent. you wish to make; then add to this enough volumes of pure water to make the total number of volumes agree with the number of the per cent. at hand.

For example, suppose you have 95 per cent. alcohol at hand and wish to make 70 per cent. alcohol, take 70 c. c. of the 95 per cent. alcohol and add to this 25 c. c. of pure water. This will give you 95 c. c. of 70 per cent. alcohol.

The following suggestions are offered especially for the benefit of laboratory students, although most of the directions will also be useful to the amateur microscopist working at home.

The microscope must always be handled below the stage and never lifted by any part above the stage, otherwise the fine adjustment may be injured. The microscope is a very delicate instrument. It must not be inclined for general work, as temporary mounts will not stay in the field unless the stage is horizontal. While working, the observer should keep the side of the microscope with the coarse and fine adjustments toward him. The microscope is not to be moved about to obtain the light. This can be obtained from almost any direction by adjusting the mirror properly. Great care must be taken so as not to run the objective down into the diaphragm or onto the cover-glass and slide. The lenses of the microscope must not be touched with the fingers. They must be wiped

only with a very clean, soft, cotton cloth or with lens paper. They must be kept scrupulously clean. The student should learn the different combinations of low and high powers immediately and how to change from one to the other without difficulty. In the directions, the  $\frac{2}{3}$  objective with either eyepiece will be called the low power, and the  $\frac{1}{6}$  objective with either eyepiece the high power.

The wiping rags should always be clean, and the slides and cover-glasses must be kept scrupulously clean. The student should learn at the beginning how to clean the cover-glasses without breaking them. To do this, take the cover-glass, moistened in water or alcohol, in the rag between the thumb and forefinger and hold it at the edges between the thumb and forefinger of the other hand. In making a mount air bubbles are to be avoided. To accomplish this, after the object has been placed on the slide and covered with a drop of water, hold the cover-glass at the edges between the thumb and forefinger and bring it down obliquely onto a needle held in the other hand, and then withdraw the needle gradually. The cover-glass will then settle down on the object surrounded by water. No water or other reagent must be on top of the cover-glass. If too much water has been put on the slide it may be removed with blotting paper. If the study of a good specimen cannot be finished in the given time, it may be preserved for a number of days by running a little fifty per cent. glycerin under the cover-glass. This will of course kill any living organism. Reagents cost money, and are not to be poured out like water. The same is true of the material for study. This is often difficult to obtain and should be used with economy, and all good surplus material returned to the receptacle from which it was obtained.

All objects studied are to be carefully figured and described. The drawings may be outlined with the 3H pencil and then finished with the 6H. If time is at hand, the drawings may be finished in India ink with a fine drawing pen. Learn how to keep the pencils sharpened to a fine point. After sharpening with the knife rub the point smooth on a piece of paper. The drawings are to be placed only on the front side of the drawing paper. The notes may be written continuously on both sides of the note paper, but are always to be taken down in ink. The plates containing the drawings should be numbered in Roman figures at the top, and the name of the plant or object written at the bottom. The separate drawings on the plate may be numbered in Arabic figures, and a proper record of them is to be kept in the notes. The notes on each plant may be numbered the same as the plate containing the drawings to illustrate it. The drawings should not be crowded, and the number should always be written below.

The diameter of the field (the white disc visible when looking into the microscope) is about six to eight inches when projected onto the table. Learn to do this by looking with one eye on the table beside the microscope and with the other into the tube. In this way the magnified image may be directly measured. The actual diameter of the area covered can easily be determined for the low powers by examining a millimeter rule. It is about  $2\frac{4}{10}$  mm. for the  $\frac{2}{3}$  objective and  $\frac{2}{4}$  ocular combination, and  $\frac{3}{10}$  mm. for the  $\frac{1}{6}$  objective and  $\frac{3}{4}$  ocular combination. Learn to keep both eyes open when taking only ordinary observations in the microscope. Be sure to use both eyes, else one will be trained for more

acute vision than the other. Make the drawings of small objects of the right proportion, and the actual size magnified. The larger ones may have to be reduced to bring them onto the paper. If the object has a definite relation to environment do not draw it upside down. It must also be remembered that motions are magnified as well as the objects themselves.

Absolute regard for the truth is the first requirement in scientific drawings and descriptions, and the qualities required for good work are accuracy, cleanliness, patience, skill, persistency, good judgment, and logical ways of thinking. The drawings should be exact in all details; the sketches may be more or less diagrammatic. The notes should be written in the best English at the command of the student. The facts should be stated in concise but complete declarative sentences, without rhetorical ornamentation. The observations must always be recorded at the time when they are taken. One's memory should not be trusted in recording scientific facts.

Finally, it must be remembered that one of the first things to be accomplished is to educate the hand for delicate manipulations. And it is also well to keep in mind that scalpels and razors are not intended for sharpening lead pencils or cutting the table, that oculars and objectives are never to be dropped, that stoppers should not be laid down on the bare table, that books and notebooks are not to be soiled by the wet and dirty fingers, that bottles and tumblers of water are not to be overturned, and that one should understand the objects studied before attempting to draw or describe them.

#### OUTLINES.

Preliminary study of the living cell, to show the general cell structure and the activity of protoplasm.

##### 1. *Philotria canadensis* (Mx.) Britt. "Elodea," Waterweed.

This is a very common plant growing submerged in ponds, creeks, etc. It will grow well for a long time if simply pulled up and placed in a covered glass jar.

1. Carefully pull off a few young leaves and mount in water. Sketch the entire leaf under low power. Make the drawing about five inches long. Describe the shape, margin, color, midrib. Are there any other veins?

2. The leaf is composed of cells. How many across the leaf? How many lengthwise? Is the leaf more than one cell in thickness? About how many cells on the upper surface?

3. Cut cross sections with the razor by holding some leaves between elder pith. How many cells in thickness, on the average?

4. Suppose the leaf averages three cells in thickness, about how many cells in the entire leaf?

5. Under high power, draw several adjoining cells, carefully showing details. (Draw the walls as represented in Fig. 1.) What is the general shape of the cells? The contents of a cell are protoplasm and sap or water. There is usually some dead food material present.

6. Notice that the protoplasm is made up of cytoplasm, nucleus, and chloro-

plasts. Where is the green coloring matter? What is the color of the rest of the leaf? The green coloring matter is chlorophyll. What is its use? Estimate the number of chloroplasts in a single cell. How many would there be in the entire leaf? How does a green plant get its food?

7. Movement of protoplasm. Describe the motion. Do not be satisfied until the rotation is very striking. The room and water should not be too cold. Does the protoplasm rotate in the same direction in all of the cells? How many seconds does it take for a chloroplast to make the round? Does the nucleus move in the cell? The active agent in the movement is the cytoplasm. The cytoplasm does not move from one cell to another.

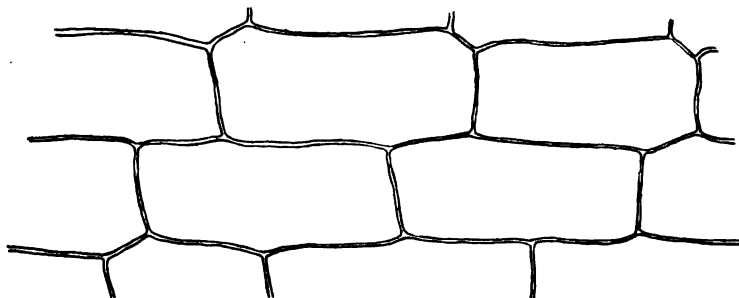


FIG. 1.

8. A cell is a small mass of protoplasm, in typical plants usually differentiated into cytoplasm, nucleus and plastids, and surrounded by a cellulose wall. The cell is the unit of plant structure. In some of the lower plants no nucleus has been discovered, and in many plants the plastids are also absent.

9. Treat a fresh leaf with alcohol. Does the protoplasm still move? Treat a fresh specimen with salt solution. What takes place? Explain the cause. Ask for an explanation or study the subject of plasmolysis in a text-book. These cells have a vacuole (water chamber) inside of the protoplasm and are normally in a turgid condition. Treat the specimen in alcohol with iodine solution. Notice the nucleus and nucleolus. Notice the large starch grains stained dark blue inside of the chloroplasts.

10. Ecological note. Does this leaf have stomata? How is it adapted to its environment?

## II. *Allium cepa* L. Common Onion.

1. Pull off the inner and the outer epidermis from a living scale of an onion. Mount in water. Compare the cells of the two specimens under low power as to shape, size, and contents. Notice the walls lined with cytoplasm; also the nuclei. Draw a number of adjoining cells from the inner epidermis. Notice the absence of chloroplasts.

2. Under high power, draw a single cell showing the wall, cytoplasm, and nucleus.

3. Study the movement (streaming) of the cytoplasm. This can usually be seen best at the ends of the cells. Notice the fine strands of cytoplasm stretching across the cell or across the corners of the cell through the large central

vacuole. Make a diagram of a cell showing the position of these streams, and indicate the direction of the flow by means of arrows.

4. Treat with a drop of iodine solution after killing the cells in alcohol. Make a careful drawing of the nucleus under high power showing the nucleoli. What is the normal number of nucleoli for each nucleus in these cells? Is the number constant? Are there any starch grains present stained blue by the iodine?

5. Why do the scales of the bulb not have chlorophyll?

### III. *Tradescantia* sp. Spiderwort.

The flowers of almost any of the wild or cultivated species of spiderwort will be found suitable. *Zebrina pendula* Schnizl., the wandering Jew, so common in window gardens and greenhouses, will also do very well.

1. Study the stamens. With a scalpel cut off some of the epidermis of a stamen filament containing some of the young hairs. Mount in water. Be careful to get the hairs wet, but do not injure them. Under low power, notice that the hair is made up of a chain of cells. Draw.

2. Study a single cell under high power. Observe the position of the nucleus; the cytoplasm, filled with small granules, lining the cell wall; and the large vacuole filled with water through which granular strands of cytoplasm stretch.

3. Study carefully the streaming motion of the cytoplasm. Are the streams constant or can you see changes going on in their position? Do some of the strands disappear entirely? Watch the position of the nucleus for some time and describe its motion. Select one that is suspended in the central part of the cell. Make a large, careful sketch of a cell showing the streaming to good advantage. Plot all the moving streams visible by focusing up and down, and indicate by means of arrows the direction of the movement.

4. Of what use is the reddish-violet coloring matter in the cells of the hairs, petals, and leaves?

JOHN H. SCHAFFNER.

Ohio State University.

## A Convenient Method for Washing, Staining, and Dehydrating Small Specimens.

It has long been known that when specimens are changed from one fluid to another of different specific gravity and allowed to remain quiet they are soon surrounded by a halo of fluid which may not mix readily with the rest. This may be obviated by keeping the fluid in constant motion.

In the case of washing in water, a large specimen may be put in any convenient vessel and tap water turned in. In the case of small objects they may be placed in a tea strainer and water allowed to pour through. But in case of staining small objects or dehydrating them this could not be done.

Various methods may be devised to keep the fluid in motion and thus prevent the halo of concentrated solution about the specimen. The writer has made use of a device which proves very satisfactory. It is so simple in construction that it may be readily made by any

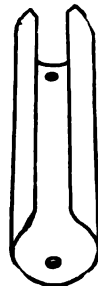


FIG. 1.

one at a trifling cost. It consists of a circular board about twelve inches in diameter, on the radii of which are fastened a number of trough-like tin pieces. These tin pieces (Fig. 1) are open at each end and just large enough to allow suitable sized cylindrical vials to be pushed in with sufficient friction to hold them securely. The specimens are placed in the vials in the desired fluid. The vials are securely corked and placed in the tin troughs on the wheel. The wheel is made to revolve slowly in a vertical plane by any desired method. This causes a complete stirring or mixing of the fluid and the specimen is very soon saturated with it.

The writer has made use of a water motor for the power and an old clock gear to reduce the speed. The balance wheel of the clock was removed and a pulley wheel put on the shaft of the escapement. This pulley connects by a belt with the water motor. The revolving disk, or wheel, is attached to the winding shaft. A moderate speed of the water motor will thus be reduced to one or two revolutions of the large wheel per minute. This is fast enough to cause sufficient mixing of the fluid without causing any damage to the tissue. The apparatus runs so slowly that the fluids are free from violent agitation. The most delicate tissue may be thus handled with safety.

JAMES ROLLIN SLONAKER.

University of Chicago.

### Oculars for General Laboratory Work.

The microscopes intended for general laboratory work, as for example the BB4, are usually furnished with the two-inch and one-inch eyepieces. The writer, as an experiment, ordered two instruments of this kind, substituting the three-fourths-inch eyepiece for the one-inch. The results were very gratifying. The one-sixth objective is not at all overtaxed by the use of the three-fourths-inch eyepiece, and the gain in magnification is considerable. The range of the four magnifications possible with two objectives and two oculars is greater and more suitable for general work. The results following the use of the three-fourths-inch eyepiece are especially noticeable in the study of minute objects, as fungus spores, bacteria, rhizoids, etc. It is the opinion of the writer that when only two eyepieces are used, for general work they should be the two-inch and the three-fourths-inch.

JOHN H. SCHAFFNER.

Ohio State University.

The present number of the JOURNAL contains a careful review of the 22nd volume of the *Transactions of the American Microscopical Society*. We learn that the 23d volume is now in press, and may be expected in the early spring. There has been a noticeable increase in the membership of the society, and the completion of the Spencer-Tolles Fund, which leaves an annual sum free for the encouragement of research, is certainly an effective influence in bringing strong members into the organization.

## A Hint for the Preparation of Internal Organs of Dried Insects.

Some five years ago attention was directed by Dr. F. Ris to the phylogenetic data afforded by a study of the gizzard-armature of nymph and imago of the same species of Dragonfly (Odonata). Dr. Ris worked on alcoholic material, so that the range of his study was limited chiefly to European species. It may be of interest to readers of the JOURNAL to learn that dried specimens may be employed for dissection and study of this and other internal organs.

In Odonate imagos, the gizzard lies within the abdomen, in any segment from the third to the seventh. If the entire abdomen be cut from the rest of the body at the base of the third segment, soaked in 70 per cent. alcohol for twenty-four hours or longer, until thoroughly softened, and slit open along one of the membranous pleura, the alimentary canal may be removed, the gizzard opened lengthwise and its muscular coat removed with fine needles. Its chitinous lining, armed with teeth, may be cleaned and mounted in balsam. This method has been employed with much success by Miss H. T. Higgins, working under my directions, as her recently published results show.\* With a little care, we were able to remove the gizzard from a pinned individual, dry the abdomen and restore it to its proper owner without impairing the usefulness of the latter as an ordinary museum specimen.

A further step may now be indicated. It is, that since some time elapses after metamorphosis before the imago ejects, through its vent, the moulted nymphal lining of its own intestine, so-called *teneral* imagos, with colors still pale and immature, may yield both nymphal and imaginal gizzard-armatures by use of the same method. This is an important gain, since it furnishes a means of obtaining knowledge of the gizzard of the unknown nymphs of various exotic groups, of which dried imagos are more or less common.

University of Pennsylvania, Philadelphia.

PHILIP P. CALVERT.

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OBSERVING THE CIRCULATION OF THE BLOOD.—Mr. F. J. Medina of Corinto, Nicaragua, has observed that the embryo of a small fish that swarms in the lake of Managua of that country, offers a most excellent means for studying the circulation of the blood in living animals. The fish lays its eggs on weeds and roots of plants growing along the lake shores, toward the beginning of the dry season, from November to March. The eggs, being very numerous and laid on different days, supply embryos in different stages of development, thus furnishing a wide range for observation. "The eye catches at a glance a beautiful sight of the whole circulatory system, and follows with delighted attention the stream of blood starting from the heart, running in the arteries and veins, and returning to the heart, whose beatings are conspicuously seen. The elongated globules of the blood are distinctly seen, forming inside the blood vessels something like a string of beans."—*Sci. Am.* 86: 2.

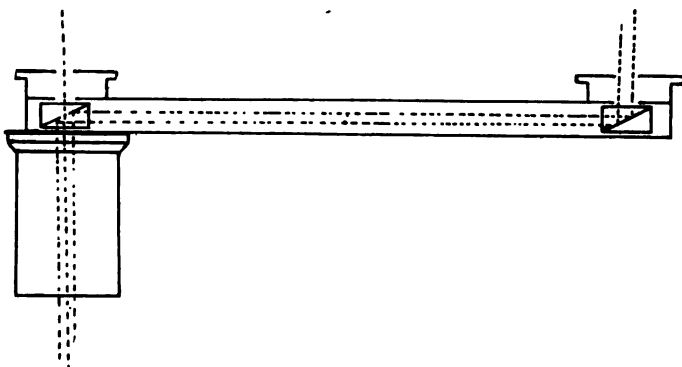
\* Proceedings, Acad. Nat. Sciences, Phila., 1901, p. 126-141, pls. ii-iv.

### A Demonstration Eyepiece—A Desideratum.

Almost every teacher has experienced the difficulty of demonstrating objects under the microscope to the student. The apparent size of objects under the microscope is so much a personal matter and the appearance of so many objects more or less confusing that the beginner is often found, after five minutes enthusiasm, to have observed the wrong object. The study of moving protozoa, bacteria, etc., increases the difficulty.

Usually some landmark is selected with which to call the student's attention to the object he is to see. Even then it takes a good deal of alternate looking by teacher and student until the particular point is satisfactorily made out. A sketch is generally used with which to point out the desired features, but this will not serve for moving objects or views that can be obtained only at intervals. Besides, the student remembers the drawing better than he does the actual object.

These difficulties could be, to a great extent, overcome by what might be called a demonstration eyepiece,—an eyepiece so constructed that both student and instructor see the image at the same time. The need of such an instrument has frequently been felt by the writer, and if it can be made practicable, no doubt others would use it. The idea, in its first inception, is here given.



A Demonstration Eyepiece.

The device consists of an ordinary eyepiece with cross-hairs for pointing out the object. A prism above the eyepiece is so placed as to reflect the image through a tube at right angles to the eyepiece to another prism at the end of the tube. This prism will reflect the image upward into the eye.

Now the instructor can point out the pseudopodia of an *amœba* as they are formed, the nucleus and the pulsating vacuole, or if the student is just beginning he may be shown the value of focusing. Such an instrument might also be used to test the student's knowledge of certain sections by having him point out and explain the structures—both instructor and student looking at the same time.

Detroit High School.

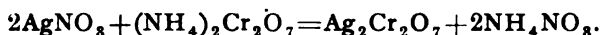
L. MURBACH.

## MICRO-CHEMICAL ANALYSIS.

## XIX.

## THE COMMON METALS.—SILVER GROUP.

*II. Ammonium Dichromate added to solutions of salts of Silver gives rise to the separation of Silver Dichromate.*



*Method.*—To the moderately concentrated test drop add a tiny drop of dilute nitric acid, then a fragment of ammonium or potassium dichromate. Dark red crystals of silver dichromate separate at once in the form of prisms, plates and aggregates belonging to the triclinic system (Fig. 77). These crystals exhibit faint dichroism when turned above the polarizing nicol.

*Remarks.*—In neutral or very concentrated solutions the crystals first form in the form of tiny rods and needles so dark colored as to appear black by transmitted light; after a time there are generally obtained, in addition to these rods, the large plates and prisms, figured above.

Silver dichromate can be recrystallized from hot water, but better results follow the use of nitric acid or ammonium hydroxide. From hot nitric acid very beautiful preparations can be obtained. According to some investigators the crystals which separate on cooling from a hot neutral aqueous solution of the dichromate precipitate are not silver dichromate, but normal silver chromate,  $\text{Ag}_2\text{CrO}_4$ .

Ammonium hydroxide dissolves silver dichromate with ease. The crystals separating from the ammoniacal solution are, according to some chemists, complex salts containing one or more molecules of  $\text{NH}_3$ . The recrystallized product separates in the form of needles, skeleton crystals, and masses resembling lichens.

In the presence of much lead the reaction often fails. Instead of the dark red salt, small yellow prisms of entirely different appearance separate. In such an event either first remove the lead with a drop of dilute sulphuric acid and then add the dichromate, or else add, immediately after the fragment of the reagent, a drop or two of dilute sulphuric acid. Usually in a short time good crystals can be obtained. The use of sulphuric acid in connection with the dichromate complicates matters, since the crystals separating in the presence of the silver sulphate formed in the reaction may be either those of the salt  $\text{Ag}_2\text{Cr}_2\text{O}_7$  or the salt  $\text{Ag}_2\text{CrO}_4$ ; the latter compound is usually formed when the amount of nitric acid is small and that of silver sulphate large. Normal



FIG. 77.

silver chromate is isomorphous with normal silver sulphate, normal silver selenate, and anhydrous sodium sulphate, all are to be referred to the orthorhombic system. Because of this isomorphism of the sulphate and chromate very interesting and instructive preparations can be obtained. Such preparations are best understood by performing the experiments outlined in the last paragraph of the *Exercises for Practice* given under this method (II). Silver sulphate separates from solution generally in the form highly refractive, transparent, colorless, rhombic octahedra, but in the presence of silver chromate these colorless octahedra increase in size, turn first yellow, and finally a more or less intense brownish red.

Mercurous salts yield with ammonium dichromate in solutions acidified with nitric acid a number of different compounds (see Mercury) varying in composition and appearance according to the conditions which obtain. There is, however, little danger of confusing these salts with the silver dichromate, though they are sometimes apt to interfere with the test for silver.

Normal potassium chromate added to neutral solutions of silver causes the precipitation of normal silver chromate; but when the test drop is first acidified with nitric acid the crystals separating probably consist of both the chromate and dichromate. When recrystallized from hot nitric acid the precipitate will usually consist of the dichromate alone. When ammonium hydroxide is the solvent employed to recrystallize the silver chromate, the compound separating is thought to have the formula  $\text{Ag}_2 \text{CrO}_4 \cdot 4\text{NH}_3$ .\*

When working with test drops acidified with nitric acid there is little danger of any interference by members of the calcium group.

In all analytical work it is safe to work on the assumption that the presence of any elements which are precipitated as chromate or dichromate in acid solution will interfere with the reaction for silver, particularly when such elements are in excess of the latter.

Large amounts of the salts of the alkalies seem to have an injurious effect when but little silver is present.

White alloys believed to contain silver can be tested for this element by drawing across them a streak of a solution of ammonium dichromate in nitric acid. The color of the streak is generally sufficient to indicate the presence or absence of silver; but if the streak of the reagent be examined under the microscope (best with an illuminating objective or some form of vertical illuminator) in the presence of silver the characteristic dark red crystals of the silver dichromate will be easily distinguished.

#### *Exercises for Practice.*

To a moderately concentrated neutral test drop add a fragment of  $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$ .

Acidify a test drop with  $\text{HNO}_3$ , then add the reagent.

Draw off the mother liquor from a precipitated test drop and recrystallize the silver salt by heating with  $\text{H}_2\text{O}$ . Try another preparation by heating with dilute  $\text{HNO}_3$ . Recrystallize a third portion of the silver compound, using  $\text{NH}_4\text{OH}$ .

\* Ladenburg, Handwörterbuch, 10: 713.

Make a mixture  $\text{AgNO}_3$  and  $\text{PbNO}_3$ , acidify with  $\text{HNO}_3$ , then add a drop or two of dilute  $\text{H}_2\text{SO}_4$  and finally a fragment of  $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$ .

Repeat the last experiment, adding this time the  $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$  first, and then the  $\text{H}_2\text{SO}_4$ .

Test several different preparations containing mixtures of the calcium group and silver.

Test a mixture of  $\text{AgNO}_3$  and  $\text{HgNO}_3$ .

Make a rather concentrated neutral test drop of  $\text{AgNO}_3$ , add a tiny crystal of  $\text{Na}_2\text{SO}_4$ . Study the  $\text{Ag}_2\text{SO}_4$ , which soon separates. Then add to the preparation a fragment of  $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$ . Note well all that takes place. If a selenate is at hand, substitute it in a new preparation for the  $\text{Na}_2\text{SO}_4$ .

Cornell University.

E. M. CHAMOT.

### A Finish for Laboratory Table-tops.

The following simple method for finishing the tops of laboratory tables gives them a good appearance and furnishes a surface not easily acted upon by alcohol and the other more usual reagents. It consists of three procedures :

I. By means of a woollen cloth apply to the freshly planed and sand-papered surface of the table a mixture consisting of equal parts of turpentine and linseed oil, using it freely, and rubbing it in evenly and well. Let it dry two or three days.

II. Dissolve in turpentine shavings of yellow beeswax until the mixture forms a jelly which, at ordinary temperature, is of about the consistency of vaseline, becoming a clear liquid when slightly warmed.

Apply this warm by means of a woollen cloth, rubbing it in as in the case of the first mixture, and let it dry over night.

III. Polish by rubbing, first, with a brick or a flat-iron covered with woollen cloth, and then with a piece of dry cloth held in the hand. The heavy object removes the superfluous wax, but leaves the surface dull, while repeated slight rubbing of the second cloth produces a slight polish or gloss.

I have not as yet observed the results of this method long enough to test its lasting power, or how far it will resist the various reagents, but think it probable that an occasional re-application of the wax and turpentine would be advisable, to keep the surface in good condition. Regarding resistance to reagents, the method is seen to consist essentially of filling the surface pores of the wood with wax, which naturally repels all aqueous solutions and which would be but slightly affected by alcohol. Turpentine or xylol will, of course, dissolve the wax, but an injury so caused is hardly noticeable and may be easily repaired by a local application of the wax solution. Inks and staining fluids seem in general to be repelled by the wax, at least for a short time, and, if wiped away within a few minutes, leave no mark.

In general appearance a table-top finished in this way compares favorably with other methods of finishing woodwork, and does not give the unfinished appearance of table-tops left in the natural state.

Smith College.

HARRIS HAWTHORNE WILDER.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### II.

#### BACILLUS TUBERCULOSIS.

The sputum should be collected in the morning, and great care should be taken to obtain a representative sample and to prevent infection from careless handling. The patient should be instructed to expectorate in a bottle or tin box, and to be sure that the sputum comes from the lungs and not merely from the throat or nose. To prevent contagion collect the sputum in a wide mouthed one-ounce morphine bottle, in which put a small shell vial with a little cotton in it, dropping thereon two or three drops of formaldehyde. The vial should be long enough that it will not lie flat in the morphine bottle, yet not so long but that a well fitted cork could be put in place above it. The vapor from the formaldehyde will sterilize the sputum, but will not impair its staining properties. Spread a thin film of the sputum, selecting a cheesy particle, on a cover-glass held with the Cornet forceps, using the inoculator for this purpose, which should be sterilized before and after using. Allow the film to dry in the air, after which pass it rapidly three times through an alcohol or Bunsen flame. Stain with carbol fuchsin, which may be purchased ready for use or be made after the following formula :

Fuchsin (basic)	-	-	-	-	-	-	-	1 gram.
Absolute alcohol	-	-	-	-	-	-	-	10 c. c.
Carbolic acid, five per cent., in distilled water	-	-	-	-	-	-	-	100 c. c.

Put on the cover-glass with a pipette all of the stain that will readily remain. Hold the cover over an alcohol or Bunsen flame and heat carefully until the stain steams. Allow the heated stain to act for three minutes, drain off surplus, but without washing proceed to decolorize and counterstain at the same time by using the following methylen blue solution, known as Gabbett's blue, which may be bought ready for use or prepared as follows :

Methylen blue, powder	-	-	-	-	-	-	-	2 grams.
Sulphuric acid, ten per cent., in distilled water	-	-	-	-	-	-	-	100 c. c.

Apply this stain to the cover-glass and allow it to act one minute, then rinse in water. If the film has a decided bluish tint it is ready for examination ; if not, the blue stain should be again applied and allowed to act a little longer, after which the cover should be again rinsed in water, then in alcohol, dried, and mounted in a drop of balsam on a clean slip. The tubercle bacilli being exceedingly difficult to stain are in this way stained a deep red with the heated carbol fuchsin. As they are very difficult to decolorize, they retain their red color in the presence of the acid of the methylen blue solution, which decolorizes and stains blue all other bacteria. In preparations stained by this method the tubercle bacilli will appear as slender, more or less curved rods of a deep reddish color. All other bacteria and tissues will appear of a deep blue color. The diagnosis may readily be made by these peculiarities in the staining of the organism.

**BACILLI WITH WHICH BACILLUS OF TUBERCULOSIS MAY BE CONFUSED.**

Bacillus of Leprosy, Bacillus of Syphilis (Lustgarten), Smegma Bacillus.

Bacillus tuberculosis and these three bacilli, when stained with hot carbol fuchsin, look very much alike. But by subsequent treatment they can be differentiated.

The so-called bacillus of syphilis is almost immediately decolorized in a five per cent. solution of sulphuric acid in water. The smegma bacillus found under the prepuce and on the vulva loses its color promptly in alcohol. Therefore to differentiate, proceed as follows: Stain in hot carbol fuchsin—decolorize in five per cent. sulphuric acid solution in water until the film appears free from color to exclude the bacillus of syphilis, then wash in alcohol to exclude the smegma bacillus. Red stained bacilli which may remain will be the bacilli of tuberculosis.

FIG. 1. Bacillus of Tuberculosis (Sputum) Stained with Carbol Fuchsin and Gabbett's Blue. Magnification 800 diameters, Bausch & Lomb  $\frac{1}{4}$ " objective Zeiss projection ocular No. 4.

FIG. 11. Same as Fig. 1. Magnification 1200 diameters as seen with 1-12" oil immersion, Zeiss projection ocular No. 4.

or leprosy. The conditions and circumstances will ordinarily eliminate one or the other. But if desired the differential diagnosis between them may be effected by the following method: Stain another prepared cover in cold carbol fuchsin six minutes, remove and decolorize in acid alcohol (nitric acid one part, alcohol ten parts). By this method the bacillus of leprosy will be stained, the bacillus of tuberculosis, the smegma bacillus, and the bacillus of syphilis will not be stained.

**METHOD OF EXAMINING CASES OF DIPHTHERIA.**

In all cases of sore throat the medical man should have in mind the possibility of diphtheria, as occasionally a case of mild sore throat may be a diphtheritic one and the contagion spread may become malignant. A microscopical examination is therefore necessary early in every case. At this stage there are so few of the Klebs-Loeffler bacilli present that the diagnosis cannot certainly be determined from the microscopical examination when made direct from the throat. In such suspected cases it is better to make a culture, and the physician should have for this purpose sterilized apparatus; but as it is not always possible to have this, and improvised methods may be made to give good results. If nothing else is at hand a pine stick with a little cotton wrapped on the end may be used as a swab which should be rubbed against the infected part of the throat, usually the tonsil. Rub rather hard so as to get some of the material on the cotton. The stick may then be inserted in a test-tube or bottle with cotton wrapped around it so as to make a stopper. This will enable the infected swab to be carried home or to some bacteriologist's laboratory without drying. Rub the infected swab on the surface of a prepared blood culture tube, which may be pur-

chased from a number of firms who make it a business to supply these tubes at a very low price, so that the physician may have a half-dozen on hand, as they will keep for a considerable length of time. This tube should be kept in a warm place for twelve hours, and if no other place is available it may be carried in a pocket in the underclothing next to the body, where it will be kept warm enough for the bacteria to grow. After twelve hours the micro-preparation should be made from the surface of the medium in the tube. Remove a portion of the growth with the loop of the inoculator, spread this in a drop of water on a clean cover-



FIG. III. *Bacillus Diphtheriae* (Culture). Stained with Methyl Violet. Magnified 1800 diameters. Photomicrograph with 1-12" oil immersion objective. Compensating Photo Ocular No. 2.

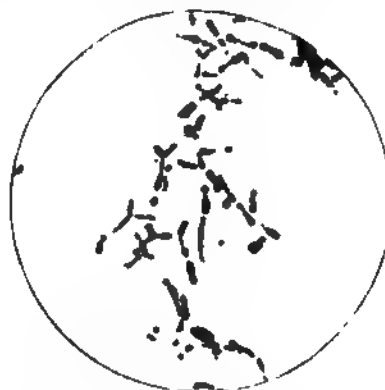


FIG. IV. *Bacillus Diphtheriae* from an old culture. Degenerative forms. Stained as above. Magnification 1300 diameters. Compensating Ocular No. 2.

glass held in Cornet forceps. Dry the film in the air and pass it three times through the flame of an alcohol lamp or Bunsen burner. Stain with methyl violet solution for about one minute, wash in water, dry between filter paper, and mount film side down in a drop of balsam on a clean slip. Loeffler's alkaline methylene blue also gives well stained preparations. The form and appearance of the bacteria will determine the diagnosis of the case should it be a typical one of diphtheria. Many cases of sore throat are due to streptococci. By this method they will be stained. In cases of mixed infection, where both the bacillus of diphtheria and streptococci are present, it is difficult to determine definitely whether it is diphtheria or not, and a second tube culture may be necessary to decide the question.

#### PNEUMOCOCCUS, Croupous Pneumonia.

Spread a thin film of the sputum on a clean cover-glass held in a Cornet forceps. Fix to the cover by passing it rapidly three times through an alcohol or Bunsen flame, apply a one per cent. aqueous solution of acetic acid one or two minutes, drain off the surplus fluid, and without washing apply the following stain, which should be freshly prepared:

Anilin oil, saturated aqueous solution	-	-	-	20 c. c.
Absolute alcohol	-	-	-	20 c. c.
Saturated alcoholic solution of gentian violet	-	-	-	22 c. c.

Staining takes place rapidly. Wash the cover in water, dry between two pieces of filter paper, and mount film down in a drop of balsam on a clean slip.

By this method differential staining of the capsule and the diplococci takes place. For ordinary diagnosis stain the prepared cover in a methylene blue, Loeffler's, or in methyl violet solution, which will show the diplococci well. By these methods the presence of the micro-organism may be demonstrated both in the sputum and in the blood.

WILLIAM H. KNAP.

Harvey Medical College.

**LABORATORY PHOTOGRAPHY.**

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

**PHOTOGRAPHING UREDINEAE WITH THE MICROSCOPE.**

The apparatus used consists of a small Zeiss microscope, projecting ocular No. 2, condenser of 1.00 N. A., biconvex lens on adjustable stand, 3-in. B. & L. objective, one-sixth Spencer oil immersion objective, made specially for use with orthochromatic plates, and an acetylene lamp.

When using the one-sixth objective the lamp is placed about four feet from the stage of the microscope, and the condenser accurately centered by taking a 1½-inch objective and projection ocular, and focusing on the edge of the iris diaphragm. The biconvex lens is then placed between the light and microscope so as to bring the image of the flame a few inches from the substage condenser, and centered by focusing on the image of the rim. The lamp is then adjusted until, on looking down the tube, the image of the flame is in the center of the image of the biconvex lens.

FIG. 1.—*Aecidium fraxini* x12.

on the stage, a piece of ground glass placed so that the image of the flame from the biconvex lens is sharply focused upon it (moving the ground glass into the focus and not disturbing the lens), and the substage condenser raised or lowered until a sharp image of the flame is seen on the object, and the glass is then removed.

Until recently I supposed that a projection eyepiece cut off all reflections and useless rays, but I find that much finer negatives can be made by using an iris diaphragm between the objective and tube of the microscope and closing the opening as far as possible without showing in the field of view, and the illustrations with this article were all made with

The slide to be photographed is placed

FIG. 2.—*Puccinia amphigena* x250.

it. It is also necessary to close the iris diaphragm of the condenser until the glass then with a look glass open, the ob-

scidia  
the  
used  
object,  
enser  
tions.

exposure of about one minute.

All developed with ortol, which I have just begun using and like better than any I have tried.

Decorah, Iowa.

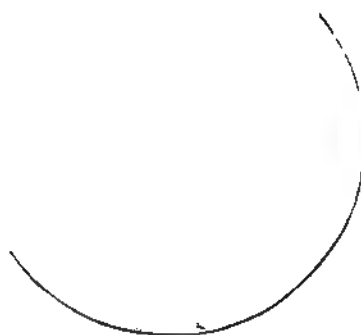


FIG. 4.—*Puccinia dochmia* x250.

E. W. D. HOLWAY.

### AN X-RAY TABLE.

This table is a device for taking X-ray pictures stereoscopically; its construction permits the operator to shift the position of the X-ray tube a measured distance horizontally or vertically, and also permits the removal of one photographic plate and the substitution of another beneath the part to be pictured without disturbing the patient; the second plate occupies precisely the same position as the first.

The table is made of hard wood, and is very solidly constructed for the sake of stability. It is six feet long, nineteen and one-half inches wide, and thirty-four and one half inches high. It is supported on eight strong wooden legs. The top of the table contains three rectangular openings, one in the center of the table and one at either end. These openings are seventeen and three-quarters inches long and fourteen and three-quarters inches wide. This corresponds to the size of 14 x 17 X-ray plates enclosed in their envelopes. Over the entire top of the table is stretched a sheet of pegamoid, with a canvas backing.

The solid portions of the table are padded with a sheet of felt slightly thicker than an X-ray plate included in its envelopes. Beneath the table, and corresponding accurately in size and situation to the openings above described, are three wooden plate carriers, which slide vertically in suitable guides. Each carrier is raised and lowered by means of strong wooden supports beneath the table arranged in the form of a toggle-joint. When the elbow of the joint is straightened the plate carriers are raised and pressed firmly upward against the pegamoid covering of the table. When bent, the plate carriers descend and permit the introduction or removal of the photographic plates, a suitable space being provided for this purpose at one side of the table. The position of the plates upon the carriers is accurately fixed by means of wooden kits of different sizes.

FIG. 1.—An X-Ray Table.

The lower limbs of the toggle-joints are pivoted beneath upon a heavy iron bar which extends the whole length of the table. The weight of the patient's body overlying the carriers is thus firmly supported. Upon the pegamoid surface of the table the position of the different sized plates is clearly marked by shallow grooves in the cloth, corresponding to the situation of the underlying plates. The part to be pictured may thus be arranged upon the surface of the table with reference to the underlying plates without difficulty. Along either border of the table, and below the level of its upper surface, three pairs of metal knobs or buttons are affixed opposite to each opening. To these buttons thin leather straps are fastened; they may be buckled across the part to be pictured, thus securing complete immobility during the exposure.

The device which holds the tube, and permits it to be moved a measured distance horizontally or vertically, is constructed as follows:

A heavy cylindrical bar of hard wood two inches in diameter, and four feet eight inches long, is affixed vertically to the side of the table opposite to the side into which the photographic plates are inserted. This bar passes through a block which may be moved, in horizontal grooves, from one end of the table to the other. The bar may also be rotated on a vertical axis, and moved up and down vertically any desired distance. It is fixed by means of a brass friction clamp. Two secondary blocks, one on either side of the first, serve as fixed points to which the vertical arm can be returned if displaced for any reason.

From the upper end of the vertical arm there extends a horizontal arm, also of hard wood, long enough to permit the X-ray tube to be suspended from it by means of a heavy wooden clamp over any part across the width of the table.

FIG. 2.—The table ready for an exposure.

Both vertical and horizontal arms are marked off in inches. The scale on the vertical arm indicates the distance of the anti-cathode of the tube from the photographic plates. The clamp for the tube hangs vertically downward from the horizontal arm, and at its lower end bears a pair of grooved jaws padded with rubber, so placed that when the horizontal arm is at right angles to the long axis of the table the X-ray tube is very firmly held, with the plane of the anti-cathode at an angle of  $45^\circ$  with the surface of the table, and with the long axis of the tube parallel with the long axis of the table. The construction of this gallows frame, as it may be called, is heavy, for the sake of rigidity. Metal should be avoided as far as may be in its construction.

Stereoscopic X-ray pictures are taken as follows: A photographic plate, in its envelopes, is inserted into the carrier; the elbow of the toggle-joint is straightened, thus raising the plates into contact with the pegamoid covering of

the table. The part of the patient's body to be pictured is arranged upon the table in proper position over the plate. The tube is then brought into position over the part, and an exposure made. The carrier is then opened, the plate removed, and another inserted. The tube is moved two and one-half inches, and a second exposure made.

These two plates, when developed, constitute stereoscopic X-ray negatives. They may be viewed at once in a Wheatstone reflecting stereoscope by transmitted light, or they may be reduced to a size suitable for the refracting stereoscope, and viewed as positives on glass or paper.

I wish to acknowledge my indebtedness to Mr. W. F. Folmer, of the Folmer & Schwing Manufacturing Co. of this city, to whose careful oversight the carrying out of the details of construction is due.

New York.

A. B. JOHNSON.

## NOTES ON THE MICROSCOPE.

### II. Early Accessories.

A comparison of the earliest accessories with those now in use shows that many of them are almost identical. Many of the old forms have fallen into disuse only to be revived as new from time to time. The concave reflecting mirror, used attached to the objective to illuminate opaque objects, known as Lieberkuhn's illuminator, was first invented by DesCartes in 1637, Lieberkuhn's adaptation dating from 1738, a century later. Both DesCartes and Lieberkuhn mounted a magnifying lens opposite the central opening, and the object was placed in the focus of the mirror.

Zahn, in 1702, figured a simple microscope with a set of rotating diaphragms of various sizes for increasing the efficiency of the lens. Subsequent makers do not seem to have made use of this improvement until about 1820, when it was revived as new by Le Rallier.

The very early microscopes were generally provided with sets of accessories specially made for them, and intended particularly for the examination of special sets of objects. Musschenbroek's microscope (1690) had with it a pair of forceps with delicate points, and a holder for the object carrier, adjustable in any direction by means of two ball and socket joints. The object carriers were metal rods, provided with one or two points, sharp, rounded or clamp like, for insertion into the object, or for holding a transparent object mounted between mica plates. In a little hand microscope by Baker (1740) we find a contrivance for holding the object identical in construction, and even in shape, with the stage forceps



Musschenbroek's accessories.

now regularly made for use with the most improved microscopes. A camel's hair brush was also considered a necessary auxiliary, as were needles mounted in wooden handles.

An important part of Marshall's microscope for the examination of the blood (1704) was a fish trough with glass bottom and metal sides. The small fish to be examined was held quiet by a metal half-cylinder placed over its body, and from which the tail projected.

Ellis's aquatic microscope (1755) was the precursor of the modern dissecting stand, which it resembles very much in general appearance. The lens with Lieberkuhn illuminator was carried on an adjustable arm. The stage was a circular frame in which a transparent plain glass plate, with black spot, placed eccentrically for use with opaque objects, or a thin watch-glass, could be placed. A projection at the side of the stage supported a detachable stage forceps for examining mineral or other solid objects on all sides, an accessory which might well be adapted to our present dissecting stands.

#### A microscopist at work.

Martin's large microscope (1780) had a mechanical stage with rectangular movements of great delicacy. An apparatus for holding "animalcules" consisted of a delicate open framework holding seven glass discs of various sizes, each provided with a glass cover held in a metal ring pivoted at the side so that the cover could be swung aside; the fluid containing the organisms being held between the two glass discs. This apparatus was attached to the stage when in use, so that each cell could be brought under the objective. Martin's microscope had no less than twenty-four objectives, all non-achromatic of course, ranging in power from four inches focus to one-tenth of an inch, each of the lower powers being supplied with a Lieberkuhn. One of the accessories furnished with Adam's Variable microscope (1771) was a hand magnifier, mounted exactly as are the watchmaker loupes of to-day.

On the whole, it may be said that the accessories of the early microscopists were few in number, as were their requirements, but the elaborateness of mounting and ornamentation would impress us at this time, when the microscope has become an instrument of such practical utility, as extremely impractical.

L. B. E.

The writer has received much interesting information regarding old microscopes now in the possession of individuals and institutions. We should be glad to have more, with photographs of the instruments, for future use in the JOURNAL.

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The meeting of the American Society of Naturalists and affiliated societies at Chicago during convocation week was a large and very enthusiastic one, although, on account of the distance to be travelled, many of the eastern naturalists whose faces have been so familiar at these meetings heretofore were absent.

Nearly everyone has at some time or other felt that the summer months were not the proper ones in which to hold the meetings of those societies composed of professional men, and the decision to meet during the midwinter recess has met with universal approval.

In addition to the interesting programs, in the various sections, nearly two hundred and fifty titles being presented, the general discussion of the relations of the scientific societies of the United States was one of great importance to the future of these organizations.

The relation of the American Society of Naturalists and affiliated societies of specialists to the American Association for the Advancement of Science was treated very ably by Dr. C. S. Minot, in his presentation, as president of the A. A. A. S.; of their proposition that the A. S. N. relinquish its paternal position toward the affiliated societies of specialists, and itself become affiliated with the larger body, in order that the whole domain of scientific work may be adequately represented in an organization whose meetings will be of such value that no one interested can afford to be absent from them, and of such magnitude as to impress the public as never before with the extent and importance of scientific work. The discussion developed the general desire for coöperation, and at the same time the need for local centers for those who are unable each year to attend the general meeting. In fact, one suggestion was to organize local societies of naturalists over the entire country, to meet every year, and with whom all A. A. A. S. members could meet without additional fees and on equal terms, and to have a national meeting every second or third year, at which all would thus find it possible to attend.

Committees were appointed to coöperate in formulating plans for future meetings. The American Association of Naturalists and the Naturalists of the Central and Western States will meet next winter, during convocation week, at Washington with the A. A. A. S.

An interesting series of papers on the Technique of Biological Projection, by Prof. A. H. Cole, Lake High School, Chicago, will be begun in an early number of the JOURNAL. This series will be particularly valuable as a practical guide to the exact methods of treating each of the principal forms of life required for class demonstration,—the mounting, anesthetization, and manipulation under magnification, with descriptions of Prof. Cole's special apparatus. This series is the result of about eighteen years' continuous work on this subject.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Zacharias, E. Beiträge zur Kenntniss der Sexualzellen. Ber. d. deutsch. bot. Gesell. 19: 377-396, 1901.

Chemical and morphological researches upon the spermatozoids of plants and the spermatozoa of animals indicate

that the cilia and spiral bands of plant spermatozoids correspond to the tail and head of animal spermatozoa. The spiral band and the head are distinguished chemically by their nuclein contents, nuclein being lacking in the cilia and in the tail. Besides many animal forms, the writer investigated the spermatozoids of *Nitella*, *Chara*, *Ceratopteris*, *Pellia*, *Polytrichum*, and others. The various forms were treated with a solution of sodium sulphate:

Sodium sulphate	-	-	-	-	-	10 g.
Acetic acid	-	-	-	-	-	1 g.
Water	-	-	-	-	-	100 g.

To this solution a little acid fuchsin was added. This sharply differentiates the nuclein containing portion from the part which contains no nuclein and shows that heads and spiral bands differ in their chemical behavior from tails and cilia. When the solution, with methyl green instead of acid fuchsin, is applied to spermatozoa of the salmon, the head, which contains the nuclein, becomes swollen while the tail and middle piece become sharply differentiated, but do not stain. The tail and middle piece, however, stain well when acid fuchsin is used. When alcoholic material of the spermatozoa of *Triton* is treated with the fuchsin containing solution, the tail and middle piece are not at all swollen, but become stained, the latter very intensely. The head appears swollen and slightly stained, but the staining may be due to a delicate covering and not to the nuclein containing contents of the head. Living spermatozoids of *Chara* and *Nitella* show the anterior and posterior portions of the band not at all swollen, but intensely stained, while the middle portion is not at all stained, but is very much swollen. Prof. Zacharias was able to satisfy himself that the middle piece of *Triton* and the salmon as well as the blepharoplasts of *Chara* and *Nitella* contain no nuclein. The term "middle piece" is variously used, but only when it is of centrosome origin is it to be compared with the blepharoplasts of plants.

In some cases he was able to determine that the portion of the male sexual cell which is derived from the nucleus of the mother cell contains a larger percentage of nuclein than the nucleus of the female sexual cell.

The investigations of botanists and zoölogists have not yet determined the nature of the influence which the sperm exerts upon the egg in normal fertilization, but evidence is accumulating and the present paper certainly adds some interesting facts in regard to the chemical behavior of the sperm cells of animals and plants.

C. J. C.

Schaffner, J. H. A Contribution to the Life History and Cytology of *Erythronium*. Bot. Gaz. 31: 369-387, pls. 4-9, 1901.

The flower in the deeply buried bulb begins to develop in early summer and by the first of October the archesporial

cell in the nucellus and the pollen mother cells in the anther can be distinguished. By December first, the pollen mother cells have divided to form the four microspores. The first division of the nucleus of the microspore takes place sometime between December first and April first. The nucleus of the archesporial cell in the nucellus does not divide until spring and, consequently, this nucleus, in which the reduction of chromosomes takes place, has a period of development extending over six, and sometimes even eight, months. The flowers are growing rapidly before the frost is out of the ground. Ripe seed is scarce, but the plant propagates very readily by bulbs. In mitoses in the bulb the spindle is bipolar, first appearing as dome-shaped caps at opposite poles of the nucleus, as described by the author for the root tips of *Allium cepa*.

Pollen grains are well developed before the frost is out of the ground. The tube nucleus stains slightly and is comparatively small, while the generative nucleus is large and is surrounded by a densely staining mass of cytoplasm which is organized into a cell of amoeboid form.

In the nucleus of the megaspore the spirem takes the form of twelve loops which break apart to form twelve chromosomes. The chromosomes present little uniformity in size and also vary in shape, but it can usually be seen that they are double. During metaphase each chromosome breaks in the middle, thus accomplishing a transverse or reducing division in Weissman's sense. The writer, however, admits that his evidence in favor of a transverse division is not conclusive, but insists that there is less evidence in favor of a longitudinal division. Multipolar spindles are frequent, but are not regarded as a stage in the development of the bipolar spindle.

Prof. Schaffner is still an ardent champion of the centrosome in higher plants and he attributes to it an essential role in the development of the spindle and also of the radiations at the poles. After the first division the daughter nuclei pass into the resting condition and in subsequent divisions the splitting of the chromosomes is longitudinal. There is no row of macrospores, but the development proceeds as in *Lilium*, *Tulipa*, and *Fritillaria*.

Fertilization was not studied, but the development of the embryo was traced in some detail. The figures create the impression that we are dealing with a case of polyembryony, but only a single embryo is formed and the resemblance to polyembryony is attributed to the behavior of the large suspensor, which is highly developed as an absorbent organ.

C. J. C.

Lang, Wm. H. On Apospory in *Anthoceros laevis*. Ann. of Bot. 15: 503-510, pl. 27, 1901.

Although apospory has been known for some time in mosses and has been discovered in ferns, the present instance

is the first to be recorded for liverworts. For the culture, young unopened sporogonia of *Anthoceros laevis* were cut into pieces about 5 mm. in length and laid upon damp sand under a bell jar. After a month the pieces were more or less decayed, but from the cut ends, and sometimes from the surfaces, small green outgrowths could be detected. A study of sections showed that in nearly all cases the outgrowths arise from single cells of the sporophyte and that their development corresponds closely to the development of the gametophyte of *Anthoceros* from the spore. Abundant evidence was secured that the growths did not come from spores in the sporogonium or from spores accidentally introduced into the culture. The limited amount of material did not permit a study of the attendant nuclear phenomena.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Heidenhain, M.** Ueber eine Paraffineinbettung mit Schwefelkohlenstoff als Durchgangsmedium. *Zeit. f. wiss. Mikros. u. f. mikros. Tech.* 18: 166-170, 1901.

The advantages of this process are as follows: (1) Carbon-bisulphid is an extremely good penetrator, a fact probably dependent on its low molecular

weight in comparison with that of the ætherial oils usually employed. (2) The greater number of the embedding processes can be carried on at the low temperatures of 41°-42° C; since at this temperature carbon-bisulphid holds a great deal of paraffin in solution. (3) This low temperature makes it possible to leave the tissue in the mixture longer without injury, thus also ensuring better embedding. (4) Carbon-bisulphid does not cause oxidation, hence easily oxidizable stains, as chromhematoxylin, can be used before infiltration.

Three bottles of mixtures are used, one of carbon-bisulphid and alcohol in equal parts; a second and third, of the liquid pure. Tissues after complete dehydration are left in each bottle for 24 hours. Well stoppered bottles are kept at the temperatures of 36°-38° and 56°-57°, respectively, and paraffin added to saturation; in case of using the higher temperature, 3-4 volumes of paraffin go into solution. But very much goes in at the lower, which is below body heat. After the tissues have been in both mixtures, they are put into pure paraffin at 55°. They are passed through two separate vessels for an hour each, to certainly remove all trace of carbon-bisulphid.

Pieces of tissue thus treated are glassy, transparent, honey-colored, and homogeneous; moreover, they cut extremely well. Even muscle cuts in sections of 4  $\mu$  in thickness with no trace of "knife artefacts." There are a few precautions to be taken in the use of this reagent. First, it is highly inflammable, and only closely fitting, ground-glass stoppered bottles should be used. None of the bottles containing either the sulphid, or the mixture of it and paraffin should be opened near a flame. Second, to avoid the extremely disagreeable smell. Experiments proved that this can be almost entirely avoided by keeping the liquid as undisturbed as possible; shaking a vessel containing it may easily be followed by an explosion of carbon-bisulphid gas. Vessels should always be lifted carefully, pieces of tissue should be put in gently, and the generation of gas is largely avoided.

After the tissues have been in the paraffin mixture for some time, the whole should be stirred by shaking moderately; but obvious precautions can be taken to avoid dangerous or unpleasant results. While the pieces of tissue are in the carbon-bisulphid-alcohol mixture they are transparent. Passing into pure carbon-bisulphid produces some clouding. At first the pieces float, but with saturation sink to the bottom.

The mixture of alcohol and carbon-bisulphid must be renewed whenever any fine grey particles appear; the glasses of pure sulphid must also be changed when similar conditions appear. On the contrary, the paraffin-sulphid mixture lasts for a long time. If the last paraffin has any sulphur in it the blocks will crumble.

A. M. C.

**Schaffer, Josef.** Grundsatz, Interzellular-substanz und Kittsubstanz. *Anat. Anz.* 19: 95-104.

The author differs from Waldeyer's recent paper (*Arch. Mikr. Anat.* 57: [1901] 335-45), especially on this

point, the dropping of the term *Cement-substance*. This term in his opinion should be retained to designate the structureless connecting substance, which unites the formed elements of the ground-substance, and it is probably of a mucoid nature. Although in small quantities, this substance occurs in many regions, for example between the fibrillæ of lamellar bones, in developing connective tissue, also in the adult tissue, making a true ground-substance for the formed elements. He also believes that this ground substance, though it may arise from the metamorphosed protoplasm, usually comes from the cells as a result of their secretory activity.

A. M. C.

**Deegener P.** Entwicklung der Mundwerkzeuge und des Darmkanals von *Hydrophilus*. *Zeit. f. wiss. Zool.* 68: 111-168, 1900.

Material used was eggs of *Hydrophilus* and *Dytiscus*. Young stages were fixed most successfully in  $\frac{1}{2}$  per

cent. chromic and picro-sulphuric solutions. The eggs may be brought at once into the fixing fluid warmed to 80°-90° C., or previously killed in hot water of the same temperature. After 2-3 minutes in the hot liquid the eggs are transferred to cold fixing fluid for 12 hours. If chromic acid is used the eggs are then washed in water, if picro-sulphuric, in 63 per cent. alcohol for 2-6 hours, and placed in 93 per cent. alcohol for preservation. Older eggs were best treated with a saturated aqueous solution of sublimate, warmed to 80°-90° for 2-3 minutes—cold saturated solution is also good—then put in 63 per cent. alcohol.

The larvæ and pupæ of *Hydrophilus* are killed in warm (52°C.) concentrated sublimate solution, then carefully cut and fixed further for 24 hours in the fluid, several times renewed. After 24 hours they are washed in iodine alcohol, and the digestive tract with its appendages is loosened. The sections are stained with alum carmin, borax carmin, or hematoxylin. For whole preparations of the mouth parts alum carmin was used. Clove oil was used for clearing the specimens.

A. M. C.

**Szymonowicz, L.** Lehrbuch der Histologie und der mikroskopischen Anatomie mit besonderer Berücksichtigung des menschlichen Körpers einschliesslich der mikroskopischen Technik. Würzburg (Stuber) 1901; 445 p. octavo; 169 figs., 52 Tfn.

At the end of his beautifully presented new text-book of histology the author devotes 45 pages to general and special microscopical technique. In a brief, clear manner he compares in

the general part the principal methods now in use for histological investigations. In the special part the particular methods applicable to different organs and tissues are considered; so that the reader is in a position to demonstrate what he has read and learned from earlier pages. The beautiful illustrations with which the book is supplied are a great aid to the understanding of preparations.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Boutan, L.** La Photographie sous-marine et les Progrès de la Photographie. Pp. 332, avec 52 Figures et 12 Pl. Paris, 1900. Schleicher Frères.

1893 by Dr. Louis Boutan. He has succeeded in photographing the ocean bottom and various marine animals at moderate depths in which divers can work with ease. He has also made a successful exposure with a camera at a depth of 50 meters, using the electric light for illuminating the screen photographed. The apparatus used in this last feat is shown in the accompanying figure. The

The marine zoological station at Banyuls-sur-Mer, France, has been the scene of a series of experiments in submarine photography carried on since

the screen *E* receives light from the electric lamps at *L*, supplied through the cable *C* by storage batteries aboard the boat. The shutter *ob* is maneuvered through the same cable by electricity. The author reviews the various attempts made to overcome the considerable difficulties, and reproduces in the plates the results thus far attained. Suggestions

are made for a simple camera for amateur submarine photographers and for work in aquaria. A brief historical sketch of photography is also given. C. A. K.

**Arnold, Augusta F.** The Sea-beach at Ebb-tide. A Guide to the Study of the Seaweeds and the lower Animal Life found between Tide-marks. Pp. xii, 429, with over 600 illustrations. The Century Co., New York, \$2.40, 1901.

**Newbiggin, Marion.** Life by the Seashore. An Introduction to Natural History. Pp. viii, 344. With 93 illustrations. Swan, Sonnenschein & Co., London, 1901.

These two books are mutually supplementary and both are indispensable for the seaside naturalist. The first deals with the commoner invertebrate forms of our Atlantic and Pacific coasts, and is a manual with systematic arrangement and descriptions of species. The second is a seaside natural history with brief keys for the determination of some of the common types of marine life and with full accounts of the habits of numerous species, and notes upon their structure. Miss Arnold's admirable book will be a welcome aid to teachers and amateur collectors at the seaside, while Miss Newbiggin's will afford a more readable account of the more interesting phases of marine life.

C. A. K.

**Prentiss, C. W.** The Otocyst of Decapod Crustacea. Bull. Mus. Comp. Zool. 36: 167-251, 10 pl., 1901.

This is an exhaustive paper dealing with the structure and function of the otocyst of the shrimp, the prawn, the crayfish, and the crab, and its development in the lobster. The shrimp, *Palæmonetes*, is easily kept in aquaria without running water and lives equally

well in salt or fresh water. It is hardy, well adapted for *intra vitam* stains and for physiological experimentation. The chitin of the otocyst wall presents great difficulties in technique. Freshly moulted specimens are more readily procured from highly-fed young animals, and in these the chitin is easily sectioned. Large antennules were decalcified in Gilson's fluid for 24 hours, or in Von Rath's platinic-osmic mixture for a week or ten days. Best cytological results were obtained with Von Rath's platinic-osmic-picro-acetic mixture followed by iron hæmatoxylin. Fine differentiations of fibre tracts were obtained with Von Rath's platinic chloride used for 3 to 5 days, followed by washing for two weeks in 90 per cent. alcohol. For peripheral and central endings of nerve fibres methylen blue was used as an *intra vitam* stain, injected in 1 per cent. solution in normal Na Cl. Upon death of the animal the nerve tissue desired was dissected out and upon reaching the proper degree of stain the tissue was fixed by Bethe's ammonium-molybdate method. Tissues thus treated should not be long exposed to xylol, as this extracts the stain. Preparations keep for about a year.

The otocyst is located in the basal joint of the antennule in all decapod crustacea. Its chitinous lining is shed at each moult. The fringed otolith hairs are borne upon one (*Macrura*) or three (*Brachyura*) sensory regions, and are attached by a thin walled basal bulb (*Macrura*) or in a cup-like depression (*Brachyura*), which affords freedom of movement. All sensory hairs are formed by matrix cells beneath the hypodermis. When first found (after ecdysis) processes from these cells extend into the shaft of the hair. In preparation for the next moult these are withdrawn and the matrix cells recede from the base of the old hair and arrange themselves around the nerve fibre for the formation of the new bristle, which has the form, at that stage, of a double-walled tube, like the withdrawn finger of a glove. The otoliths are grains of sand taken in from the exterior and removed after each moult. In the absence of sand, iron filings or organic matter may be placed in the otocyst. Tegumentary glands in the wall of the otocyst secrete the cement which fastens the sand to the otocyst hairs. The otocyst hairs are each innervated by a single nerve element, which terminates in the base without branching. The olfactory hairs, on the other hand, receive fibres from 100 or more ganglion cells, which terminate free and at some distance within the cavity of the hair. The central terminations of the sensory fibres are at the posterior end of the brain, and are not directly connected with ganglion cells. The author's results thus fall in line with the neurone theory. The sensory ganglion cells are bipolar, and the nerve fibres possess a perifibrillar matrix which forms the varicosities on the fibres with specific nerve stains. The shrimp-like *Decapoda* have also a nucleated myelin sheath along the greater part of the nerve fibre. The otocyst arises as an invagination of the dorsal ectoderm of the basal segment of the antennule, becoming functional at the fourth moult. Structurally the otocyst of the *Decapoda* may be roughly compared to the utricle of the vertebrate ear; the sac of *Palæmonetes* to a single isolated ampulla and its sensory cushion to a crista acustica. The closed otocyst of *Brachyura* with three sensory regions and no otoliths approaches more nearly the utricle of higher vertebrates. Experimental evidence is offered to prove that the otocyst does not function as an auditory organ. It is rather an organ of equilibration, sharing this function with the eyes and tactile bristles. In free-swimming decapods it is the most important static organ.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Parker, G. H. and Arkin, L.** The Directive Influence of Light on the Earthworm *Allolobophora foetida* (Sav.), Amer. Jour. Physiol. 4: 151-157, 1901.

erally stimulated by light, shows that (a) this form is negatively phototactic, and (b) the intensity of the directive influence depends on the part of the body stimulated. The directive influence was measured by the percentage of head movements away from the light, as compared with those towards the light or indifferent, after deducting the number of cases of turning away due to other stimuli than light. When the whole length of the worm was illuminated on one side, 26 per cent. of the head movements were away from the light; 10.2 per cent. of the movements were away when the anterior third of the body only was illuminated, and 1 per cent. when only the posterior third was illuminated.

R. P.

**Godelmann, R.** Beiträge zur Kenntniss von *Bacillus Rossii* Fabr. mit besonderer Berücksichtigung der bei ihm vorkommenden Autotomie und Regeneration einzelner Gliedmassen. Arch. f. Entwicklungsmech. 12: 265-301, Taf. VI, 1901.

of the peculiar group of orthopterous insects, the Phasmidæ. To test the organism's power of regeneration after injury, parts of the legs and the abdominal segments were removed by operation. It was found that the regeneration was in general more complete the farther distal the cuts were made. In case of the limbs no direct regeneration was observed following cuts made proximal to the tibia. Instead, autotomy ensued and this was followed by regeneration. In no case after operation were more than four tarsal joints regenerated, while the normal number is five. The different pairs of legs showed differing degrees of regenerative power. The most complete direct regeneration was found in the middle pair, less complete in the anterior pair, while in the posterior pair no direct regeneration following injury was observed, any injury causing instead autotomy. Regeneration of a part of the last abdominal segment was observed in one case. The regeneration of the legs after autotomy was more complete than that following injury. Here in several cases a complete pentamerous tarsus was regenerated.

Autotomy always occurs at the point of junction of the trochanter with the femur. Across the leg at this point is developed a diaphragm consisting of loosely arranged, spindle-shaped cells evidently derived from the hypodermis. The muscles of the femur have their origin just below this diaphragm in the

A statistical study of the direction of the movements of the anterior end of the common earthworm, *Allolobophora foetida*, when the organism is unilat-

The author has made a very thorough study of the interesting process of autotomy or self-amputation of limbs, and in connection with this the regeneration in *Bacillus Rossii*, a representative

femur itself instead of in the trochanter, as is usually the case in insects where autotomy does not occur. The actual separation of the appendage takes place in one of two ways, depending primarily on the strength of the stimulus causing it. Strong stimuli cause a sudden breaking of the limb at the preformed place, provided it is in a fixed position so as to make this mechanically possible, while weaker stimuli more often cause a slower separation resulting from histolytic processes. In both cases the wound is immediately closed and bleeding prevented by the cells of the diaphragm becoming tightly packed together. That the autotomy is a purely reflex phenomenon is proven by the fact that it can be induced in a decapitated animal exactly as well as in a normal. A very full bibliography of the subject is appended.

R. P.

**Jennings, H. S., and Crosby, J. H.** Studies on Reactions to Stimuli in Unicellular Organisms. VII. The Manner in which Bacteria react to Stimuli, especially to Chemical Stimuli. *Amer. Jour. Physiol.* 6: 31-37, 1901.

The authors find that certain bacteria respond to stimuli by a reaction of a reflex type, comparable to the "motor reflex" of the flagellate and ciliate infusoria previously described by Jennings. The "motor reflex" of the bacteria studied was found to be merely movement in the opposite direction to that taking place before stimulation. For example, a specimen of *Spirillum* when stimulated swims straight backward away from the stimulus, and then after a time swims straight ahead again. This was found to be the method of reaction to chemicals; no orientation is to be observed and collections are formed in solutions of optimum concentration as a result of this "motor reflex" in essentially the same way as are the collections of infusoria.

R. P.

**Brasch, R.** Die Anwendung der physikalischen Chemie auf die Physiologie und Pathologie. Wiesbaden (J. F. Bergman). Pp. 202, 1901.

For some time a need has been felt for a work which would digest and present in a not too abstract form those results of physical chemistry which may be brought into relation with, and used in biological work. The nearest approach which has been made towards fulfilment of this need is to be found in the present work. The principal objection which can be made to the book is that it develops the subject from the standpoint of pure human physiology, yet its treatment is on the whole so broad that this is not at all a serious drawback.

The work is divided into three main parts as follows: I. "The Physical Chemistry of the Salts in the Human Organism," containing a clear and simplified discussion of the laws of solutions, ionic dissociation and chemical change as based on the modern physico-chemical theory. In this section is also taken up the subject of the chemical composition of the organic salts of the organism and their relation to the cells and tissues. Part II deals with "The Oxidation Process in the Human Organism," while Part III is devoted to "The Energy Relations of the Human Organism." Here the author takes up the general question of how the organism gets its available energy during metabolism and develops a quantitative chemical theory for the process.

In concluding the author points out the possibilities for research in physi-

ology which are opened by the new chemistry, and deprecates the fact that the discussion of so many of the problems must now be purely theoretical, while experimentation along the lines indicated by the comparatively small amount of work which has been done in applying physico-chemical laws and methods to physiological problems, would place them on a sound basis of fact. While a considerable portion of Brasch's work is necessarily theoretical, from the very fact that so little experimental work has been done on the subject, it will nevertheless be found very suggestive and helpful both to the teacher and the investigator.

R. P.

**Poptelski, L.** Ueber das peripherische reflectorische Nervencentrum des Pankreas. Arch. f. d. ges. Physiol. 86: 215-246, 1901.

The author finds that the pancreas can function after complete separation from the central nervous system, this reflex

activity being mediated by local nerve centers in the organ itself. He concludes that the central nervous system functions in unifying and regulating the independent activity of the different organs of the body.

R. P.

**Oswald, A.** Ueber die chemische Beschaffenheit und die Function der Schilddrüse. Strassburg (Karl J. Trübner), 1900. P. 61. (Price 1 Mk. 50 Pf.).

A technical chemical study of the thyroid with a brief discussion of its functional importance and method of activity.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt, Harvard University Medical School, Boston, Mass.

**Wright, J. H.** Eine schnelle Methode zur dauernden Aufbewahrung gefrorener Schnitte. Cent. f. allg. Path. u. path. Anat. 12: 634, 1901. Also Mallory and Wright, Pathological Technique, Second Edition, p. 417, Phila. 1901.

The rapid method devised by Wright of making permanent mounts of frozen sections is a distinct advance over the procedure advocated by Cullen. Working with a good microtome and a sharp

knife, Wright and his pupils have made preparations fully equal to paraffin or celloidin sections.

1. Place the specimen in a ten per cent. solution of formalin for two hours or more. If an immediate diagnosis is desired, the specimen may be boiled in the same solution, in a test tube, for two or three minutes. Naturally, the slower method gives better histological detail. The bit of tissue should not measure over five mm. in thickness.

2. Wash in water.

3. Cut frozen sections. The carbon-dioxide freezing microtome is the most satisfactory. The blade of a carpenter's plane mounted in a wooden handle makes an excellent microtome knife. The sections should be as thin as possible. In order to obtain sufficiently thin sections the knife must be very sharp. It should be given a smooth edge by rubbing on a razor-strop.

4. Float the section on the slide and arrange it smoothly, removing superfluous water.

5. Cover the section with a sheet of smooth cigarette-paper, and then press on this, over the section, a pad of soft, smooth filter-paper, the surface of which has been moistened with ninety-five per cent. alcohol. On removing the pad of filter-paper, the cigarette-paper, which remains in contact with the slide and the section, is carefully stripped off, leaving the section adhering to the slide. The cigarette-paper prevents the fibers of the filter-paper from adhering to the section and marring its appearance.

6. Cover the section sticking to the slide with absolute alcohol for about thirty seconds and drain.

7. Flow over the section and the adjacent surface of the slide a very thin solution of celloidin in equal parts of absolute alcohol and ether. Drain off immediately. The celloidin should form a coating so thin as to be invisible.

8. Flood the slide with ninety-five per cent. alcohol, and then at once immerse it in water for ten seconds. This hardens the thin film of celloidin and prevents the section from curling and leaving the slide during the subsequent manipulations.

9. Stain with hæmatoxylin, or any other stain or combination of stains. The methods of staining tubercle bacilli in paraffin sections are applicable to sections obtained by this method.

10. Dehydrate in ninety-five per cent. alcohol followed by a little absolute alcohol. Unless care is exercised, the absolute alcohol will cause loosening of the section by dissolving the celloidin.

11. Clear with oil of origanum.

12. Mount in Canada balsam.

This method has a wide field of application. It is especially serviceable in making microscopical diagnoses of surgical material during operations. Fastening the section smoothly to the slide not only prevents distortion of the section by dehydrating and clearing agents, but it facilitates the various manipulations and it saves time. By this method good smooth sections, stained, cleared, and mounted in balsam, may be obtained within a few minutes after the specimen is received.

The fixation of the material before the cutting of the section is essential. If it is not done the section will adhere to the cigarette-paper in the process of pressing it on the slide. Alcohol or Zenker's fluid may be used instead of formalin. In either case, however, the specimen must be thoroughly washed in water to remove the fixative before it can be frozen.

J. H. P.

Williams, H. U. The Frequency of Trichinosis in the United States. *Journal of Medical Research*, 1: 64, 1901.

In the pathological department of the University of Buffalo, samples of muscles secured at 505 unselected autopsies on adult human subjects were examined microscopically for trichinellæ (trichinæ). They were found in twenty-seven cases, or 5.3 per cent.

Of the muscles examined the diaphragm was most frequently and most extensively affected. When it was not possible to examine fresh specimens the muscle was preserved in a weak solution of formaldehyde. This served its purpose well, as the worms appeared very distinct; but the muscle was rendered tough and somewhat difficult to compress. The lesions varied greatly in extent. The infection was sometimes so slight that only one or two worms were discovered.

Eosinophiles were not found in the vicinity of the encapsuled trichinellæ. Plasma cells occasionally appeared in or about the capsules, and sometimes there was a new formation of elastic fibers.

J. H. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, *Yale University.*

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Yale University, Middletown, Conn.

**Brehme.** Ueber die Widerstandsfähigkeit der choleravibrionen und Typhusbacillen gegen niedere Temperaturen. *Arch. f. Hyg.* 2: 320, 1901.

The author has attempted to verify the position that has been taken by some bacteriologists recently, that, whereas freezing does not absolutely destroy pathogenic organisms, it practically destroys the danger of infection from some of them. He experiments with the cholera and the typhoid bacillus. His method was to make bouillon cultures in test tubes, and to immerse them in a mixture of ice and salt, the mixture being renewed twice, daily. To prevent the water from entering the tubes, they were closed with a layer of paraffin. The temperature was determined by a small thermometer placed inside the test tubes. His general conclusion verifies the position taken by Sedgwick and Park, that rapid freezing destroys the vast majority of typhoid and cholera bacilli. His conclusions, in general, are that the number of bacilli diminishes very rapidly in the first few days after freezing, but that a few individual bacteria may remain alive for some time; in the case of cholera, for 57 days at  $-16^{\circ}$  C, and, in the case of typhoid, for 40 days. The number, however, that remain alive for any length of time is very small.

The author then attempts to determine whether the few bacteria left alive after such long freezing have a special resisting power against cold. He cultivates these in bouillon at a low temperature, and then tests them again by freezing; but he does not find that they have any greater resisting power than the original cultures.

The paper is valuable, in confirming an extremely important conclusion relative to the danger of the use of contaminated ice. H. W. C.

**Nakinishi.** Ueber den Bau der Bakterien. *Cent. f. Bak. u. Par.* II, 30: 97-190, 1901.

This somewhat extended article is a decided contribution to our knowledge of the structure of bacteria, which has ever, since the first study of these organisms, been a topic of constant research and speculation. The especial value of the author's contribution is due to the employment of a new method of staining and study. The method is a modification of that adopted by Bizzozero for the study of blood. As applied to bacteria, it is, briefly, as follows: A saturated solution of methylene blue in water is placed upon a perfectly clean slide and then allowed to dry. Some of the coloring matter is rubbed off until a faint sky blue tint is left upon the slide, due to the solidified methylene blue attached to the glass. Upon this dried coloring matter is placed a drop of the bouillon containing the bacteria to be studied. The whole is covered with a cover-glass and examined with a microscope. The water dissolves the methylene blue, which then slowly stains the bacteria, producing a differential stain superior to that

obtained in any other way. With this method the author has studied a long series of bacteria, including coccus, bacillus, and spiral forms, spore bearers and non-spore bearers. The details of this work cannot be given here, but the most striking feature is the demonstration, in every species of bacteria, of a distinct central body surrounded by a clearer, less staining material, on the outside of which is a thin sac. The author is quite convinced that the central body is a *nucleus*, that the surrounding material is *cytoplasm*, and the sac corresponds to a *cell wall*. The evidence that the central body is a nucleus is not based wholly upon its uniform presence, but also upon the fact that, when the bacteria divide, the division is preceded by a lengthening and then a division of the nucleus into two, one of which remains in each of the resulting cells of cell division. The division of the cell is thus almost identical with the division of a typical nucleated cell. The author finds, further, that when spores are formed, they are formed around nuclei, so that each contains in its center one of these bodies. In the fully formed spores the nuclei are no longer visible, but the study of the growth of the spores demonstrates the presence in the center of a nucleus. The author, in short, takes the position that a bacterium is a typical cell with the same parts that are found in larger cells of higher organisms. The work is very carefully done, and both the work and the figures give evidence that the author has been sufficiently careful to avoid errors. This paper must be regarded as a very large contribution to the theory that bacteria must be considered as typical cells.

H. W. C.

Hammerl. Ein Beitrag zur Zuchtung der  
Anæroben. Cent. f. Bac. u. Par. I, 30:  
658, 1901.

The author has added a few practical suggestions to the somewhat vexed question as to the best methods of inves-

tigating anærobic bacteria. He points out that the irregularity in the results obtained by different investigators is due to the uncertainty and errors of methods and, in considerable degree, to the fact that the methods employed do not totally eliminate oxygen. To remove these errors is the purpose of the work done by the author. He uses a solution of methylene blue in the culture medium, to determine whether the last traces of oxygen are removed; for when the oxygen is wholly removed the methylene blue is completely decolorized, whereas, if a trace of color remains, it indicates the presence of oxygen. Various methods of culture, previously used, proved by this test to be unsatisfactory. The plans which he adopts himself are, essentially, as follows: He uses, as a deoxidizing agent, a solution of  $\text{NH}_4\text{SH}$ , prepared in a special way described in the article. This material, placed in a culture medium, does not prevent the bacteria growth, but absorbs every trace of oxygen, as indicated by the methylene blue test. By mixing a certain amount of this material with his culture medium, and then placing his Petri dishes in an atmosphere of hydrogen, or one in which oxygen has been extracted, he obtains plates which contain absolutely no oxygen. He describes two methods of using these plates, one of which depends upon an atmosphere of hydrogen, and the other upon extracting the oxygen with pyrogalic acid. The new methods suggested by the author are, therefore, the use of  $\text{NH}_4\text{SH}$ , as a deoxidizing agent, and of methylene blue as a test of the completeness of the deoxidization.

H. W. C.

## NEWS AND NOTES.

AMERICAN MICROSCOPICAL SOCIETY.—The twenty-second volume of the Transactions of the American Microscopical Society, which has recently been issued, contains the papers and proceedings of the twenty-third annual meeting of the society, held in New York City on June 28th, 29th, and 30th, 1900. The volume consists of two hundred twenty-eight pages and thirty plates. Of the thirteen papers, nine are upon subjects which are more or less limnological in character.

Following the usual custom, the volume opens with the president's address, the subject on this occasion being "The Detection and Recognition of Blood." The author, Dr. A. M. Bleile of Columbus, Ohio, draws attention to the extreme importance, especially in certain legal proceedings, of being able to determine the presence of blood as distinguished from other substances of similar color, and to demonstrate whether it is from a human being or from one of the lower animals. As there are only two characteristics, viz., red corpuscles and hæmaglobin, by means of which blood can be distinguished from all other substances, the tests are necessarily limited to proving the presence of one or the other of these elements. The red corpuscles are so easily distorted and shrunken by exposure to the air and it is so difficult to restore them to their normal size and shape, although several methods have been claimed by their inventors to be satisfactory for this purpose, that no reliable microscopist would attempt at the present time to identify corpuscles of human blood by their microscopic characters alone, especially since it is known that in their measurements and contour the red corpuscles of several of the domestic mammals are so much like those of man that the various kinds cannot be positively separated by these features alone. If the remains of red corpuscles are found the nature of the fluid is at once shown, but in old stains it is very difficult to restore their bodily shape even though the color of the stains may be characteristic. Recourse is then made to the coloring matter, hæmoglobin, itself. The most reliable chemical test for this substance is Teichmann's, directions for which will be found in almost all text-books on histology, but one of greater delicacy and more certainty requires the use of the spectroscope. The reliability of the spectroscopic test is not, in the author's experience, impaired by the age of the stain, the influence of heat, nor the effect of strong chemicals and preservative fluids. The test is made as follows: After an attempt to find red corpuscles, and with or against success in this direction, without wasting further time or material which may be at disposal in small quantities only in a search after the less delicate hæmoglobin or the hemin crystals, so liable to fail, the substance is to be at once treated with KHO solution (5 per cent.), using heat if necessary, to effect solution, then adding pyridin ( $\frac{1}{10}$  its volume), and then  $(\text{NH}_4)_2\text{S}$ , and observing the spectrum. Where a small stain on a thin fabric is the object of study, it can be placed on a thin cover-glass, moistened with a drop of KHO solution and pyridin. After some minutes a

drop of  $(\text{NH}_4)_2\text{S}$  is to be added, the preparation inverted over a hollow ground slide, sealed with oil, and placed under the microscope, when the spectrum will show the hemochromogen band, disappearing on exposing the preparation to the air, if blood is present. The band lies midway between the D and E lines. In this way can blood be detected, with a 15mm. layer, in dilution of 1 of blood to about 20,000 of water, or, with a 40 mm. layer, 1 in 40,000, involving of actual blood about 5 to 100,000 c. c. The test can be safely made with no other optical apparatus than a small, direct vision, pocket spectroscope, which can be inserted into the microscope in place of the eyepiece, and a  $\frac{2}{3}$  or  $\frac{1}{2}$ -inch objective. It is not to be assumed, of course, that even the spectroscope accomplishes the now impossible feat of distinguishing human blood from that of other mammals.

In a paper on "Some Advantages of Field-Work on Surface Water Supplies," Horatio N. Parker discusses the desirability of making periodical examinations of water supplies, for the purpose of determining any change from the normal in clearness, bacterial content, color, efficiency of filter beds, and other factors which may affect the purity, portability, and appearance of water used for domestic purposes. The author describes how the changes arise through natural or human agencies, and indicates, in a general way, the remedies and their mode of application.

"The Work of Mt. Prospect Laboratory of the Brooklyn Water Works" is the title of a paper in which Mr. George C. Whipple, biologist and director, describes the construction, equipment, and work of the leading water works laboratory in America. The sources of the water supply of the city of Brooklyn are so numerous and so varied in character that it was found several years ago that its proper management would require much analytical work, both chemical and biological. The laboratory was built in 1897, and has been constantly in active operation since that time. The building contains rooms devoted to work in chemistry, biology, and physics, as well as one for general purposes. The staff consists of one biologist and director, one chemist, one assistant chemist, and three assistants. The amount of work accomplished may be judged from the following statement: "The regular routine includes the bacteriological examination of three samples of water from the Ridgewood pumping station and from a tap in the city, collected daily; the complete physical, chemical, and biological examination of nine samples from the distribution system, collected weekly; the physical, biological, and partial chemical examination of twenty-four samples from the supply ponds, collected weekly, with complete chemical analyses monthly; the complete examination of nineteen samples from driven wells, collected monthly; and the complete examination of twenty-one samples from the private water supply companies of Brooklyn and from the water supplies of the Borough of Queens, collected quarterly. In addition to these regular samples, many extra samples are taken at various times and places, as occasion requires. During the two and a half years that the laboratory has been in operation this schedule has resulted in the analysis of more than six thousand samples, as follows:

Samples received from July 12, 1897, to April 1, 1900	-	6471
Physical examinations	- - - - -	5025
Complete chemical analyses	- - - - -	2562

Partial chemical analyses	-	-	-	-	-	1049
Microscopical examinations	-	-	-	-	-	4688
Bacteriological examinations	-	-	-	-	-	5230
Tests for bacillus coli communis	-	-	-	-	-	2630

University of Rochester.

CHARLES WRIGHT DODGE.

(Continued.)

A METHOD OF PREPARING FISH FOR DISSECTION.—In a recent paper (Die Vorfahren der Schollen. Bull. de l'Acad. Impér. des Sci. de St. Petersburg. V Ser. Bd. XIV, No. 3, 1901. Pp. 315–450. Taf. II.) Thilo describes a method of preparing fish (Pleuronectidæ) for dissection or museum exhibition which appears to have given very good results, and will doubtless prove of wide applicability. The procedure was in detail as follows :

(1) The heart and viscera were removed from all fish which were to be used for the study of skin, bones, or muscles.

(2) The specimens were placed for some hours in a solution of 5 parts soda in 100 parts water, in an ice-chest, in order to remove the slime. They were then thoroughly washed with soap and water and rinsed in clear water.

(3) The specimens were then put into formalin (100 parts water, 2 parts formalin) for a week.

(4) After removal from the formalin the specimens were left for one to two weeks in glycerin which had been boiled for about ten minutes and then allowed to cool.

(5) At the end of this time the specimens were removed from the glycerin and hung up in a dry, airy place for one to two weeks.

(6) The fish were then put up till desired for work in covered tin or glass receptacles without any preserving fluid of any sort, the dishes merely containing air.

Thilo says that fish so prepared are entirely odorless, and that the joints are movable and the muscles well preserved for dissection. Small muscles, like the eye muscles, will not dry up for some time, even when completely exposed. It is stated that well preserved alcoholic material may be prepared in the same way with formalin and glycerin with good results. It seems possible that the method may be found useful on other material than fish.

R. P.

## Books Received.

**DaCosta, Clinical Hematology.** A Practical Guide to the Examination of the Blood with reference to Diagnosis. By John C. DaCosta, Jr., M. D., Assistant Demonstrator of Clinical Medicine, Jefferson Medical College; Hematologist to the German Hospital, etc. Containing 8 full-page colored plates, 3 charts, and 48 other illustrations. Octavo, 450 pages. Published by P. Blakiston's Son & Co., 1012 Walnut St., Philadelphia, 1901. Price, \$5.00 net.

**Parsons, Elementary Ophthalmic Optics.** Including Ophthalmoscopy and Retinoscopy. By J. Herbert Parsons, B. S., B. Sc., F. R. C. S., Curator, Royal London (Moorfields) Ophthalmic Hospital. Large 12mo., 162 pages. Published by P. Blakiston's Son & Co., 1012 Walnut St., Philadelphia, 1902. Price, \$2.00 net.

# Journal of Applied Microscopy and Laboratory Methods

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## Laboratory Equipment for Beginning Course in Zoölogy.

The question of a suitable equipment for large laboratory classes in elementary zoölogy is often a most serious and perplexing one. Not only is it difficult to find the pieces of apparatus already made, but even when purchasable, the attendant expense makes them unavailable in many cases where large numbers are required. In nearly every laboratory these difficulties have been met and solved more or less satisfactorily, usually by designing such apparatus as can be made in local shops. Fortunately, the requirements of such elementary courses are not exacting, and the improvised apparatus may serve as well as the most expensive sorts. In many cases, even, the pieces are more directly suited to the work required of them than are any that may be bought.

Such a set for the individual student, evolved in actual practical work, is described here. Aside from dissecting pans and instruments, it consists of two pieces; one, an easel; the other, a standard for the support of lenses, etc. The easel is merely a piece of soft pine or poplar board 5 x 6 x  $\frac{1}{4}$  inches, supported behind by a piece of bent wire attached by small staples. Crude and simple as this is, it ensures better work from the student at a much less degree of personal discomfort than is otherwise possible. Since the style of drawing usually required of beginners is that known as orthographic projection, it becomes necessary to view the specimen from directly above each part drawn. If no support is provided, the student either lays the specimen upon the table and endeavors to look down upon it, or he props it against books or other objects so that it may be observed more easily. In either case the process is time consuming and troublesome.

By the use of the adjustable easel, much trouble and eye strain is avoided. The specimen, a crayfish for example, is pinned to the board against a suitable shade of paper for a background, the appendages are arranged and secured to the board, which is then erected at such an angle that the line of sight falls upon it normal to the surface. In this position the animal is well lighted, is easily measured, and the tendency to introduce perspective in the drawing is minimized.

When a lateral view is desired, the specimen is pinned to the top of the board near one side, the abdomen is flexed in a natural manner and fastened to the side, the appendages are brought down and secured, and the easel adjusted at the proper angle. It is not difficult to draw the animal when thus mounted, for a proper view is easily obtainable, and the edges of the board serve as guide lines from which to measure.

For external views and many dissections, the easel is very useful, but when small parts are studied, better results are otherwise obtainable. The lens support above referred to here comes into use. It is made by taking a piece of brass rod  $\frac{3}{16}$  of an inch in diameter by 10 inches in length, rounding one end with a file, and splitting the other in the center for an inch with a saw. Two holes are drilled through this end at right angles to the split, and then, after heating, the halves are bent out until the flat surfaces lie in one plane. By means of rivets passing through the small holes, the rod is secured in the middle of a tin ointment

Some improvised apparatus for work in elementary zoology.

box lid about three inches in diameter, which, in turn, is filled with melted lead. The standard thus produced is very firm and stable and occupies little room.

The lens holder attaching the magnifier to the standard is made by taking suitable brass or galvanized iron wire and forming on one end a loop of a proper size to hold the lens, and on the other a close spiral of about four or five turns whose inner diameter is very slightly greater than that of the brass rod in the standard. Two of these are conveniently formed at one time by winding a spiral of eight or ten turns in the middle of a piece of wire twice the length of the desired support. This is then cut through the center and rings formed at the free ends for holding the lenses. It is advantageous to bend the support down-

wards so that the lens may be lowered over the edge of the dissecting pan. A lens thus supported may be swung around over a large specimen, and is conveniently focused by sliding the spiral up and down the brass rod.

This apparatus, by the addition of another lens support, serves an excellent purpose in the examination of small parts and dissections, and makes the use of the microscope much easier for the beginner. In making use of the apparatus for this purpose, it is arranged as follows: Upon the ring of the lower support is placed a piece of non-drying modeling clay (to be purchased of dealers in art and laboratory supplies). If the parts are to be examined dry, they are pressed down into the clay and arranged as desired; if they are to be immersed in water, a depression of suitable dimensions is made, and in the bottom the parts are secured. Water is now poured into the improvised pan and the specimen is ready for observation. Should specimens transfixed by pins be used, they are easily fixed and oriented in the clay. The holder is elevated to a convenient height above the table, the lens is focused, and the observer may then examine the specimen with one eye and, without moving the head, make the drawing.

The modeling clay previously mentioned is useful in many ways. When irregular objects are to be held in position, either upon the table, easel, or wire support, they may quickly and easily be secured by a piece of the clay. Small fragile structures, such as the mouth parts of insects, are readily mounted in any position by pressing them into the surface of the clay. Numerous other uses suggest themselves in practical work which need not be mentioned.

Aside from the two pieces of apparatus described, nothing more is required for class use except dissecting pans and instruments. The former should be of different sizes and may be made by pouring melted paraffin into suitable tin pans. It is usually desirable to have projections of some sort in the bottom to anchor the paraffin. For many purposes a black background is desirable, and this is obtained by mixing lampblack with the melted paraffin. Small pans may be made by using the bodies of the ointment boxes, the tops of which were utilized as the bases of the lens standards. Small pasteboard boxes thoroughly soaked in melted paraffin are light and convenient and last well.

Improvised dissecting instruments, except needles, are not to be recommended. Excellent ones, perfectly adapted to their purposes, may be purchased at reasonable prices, and are always to be preferred.

Some applications of the easel and lens supports are illustrated in the accompanying reproduction of a photograph.

University of Kansas.

C. E. McCLUNG.

## A Convenient Press for Obtaining Small Amounts of Plant Juice.

It is often necessary in the physiological laboratory to obtain a small amount of juice or sap from either leaves, stems, or roots of plants or to prepare an extract of material from some part of a plant, either for chemical analysis or physical measurements.

The extract should of course be free from any outside source of contamination, hence the method of obtaining it is highly important. For obtaining large amounts of extract, the common tincture presses are of service, but where a small amount or part of a plant is all that can be used, or where only a small amount of juice or extract is desired, the tincture press is of no particular service.

In the course of a special investigation, the author found it necessary to devise an apparatus for extracting a small amount of juice or sap from parts of plants. Since some little difficulty was experienced in finding a suitable apparatus, it will be described here, as it may be of service to others.

Fig. 1. Press for obtaining plant juice.

For the power, the frame and screw of a medium sized tincture press were employed as shown in the diagram. The wooden block (a) carries a 50 mm. porcelain funnel (b) with vertical sides and perforated bottom. The opening in the block was made of the same form as the funnel, so that the funnel would be held firmly in position and would also stand a considerable amount of pressure. The pressure is applied by means of the screw on the wooden disc (c), which is made to fit loosely in the end of the funnel. The parts of the plant from which the extract is to be made must first be cut or ground fine. This can easily be accomplished by means of a small Enterprise food-chopper. By this means it was possible to obtain, with comparative ease, a few cubic centimeters of extract or a larger amount if desired.

F. D. HEALD.

Parsons College.

### Ink for Writing on Glass.

During a recent visit to some of the foreign laboratories I found in use in the laboratory of the University of Berne an ink for marking on glass which is so satisfactory that I have adopted it for use in the laboratory with which I am connected. It consists of a mixture of three parts of a 13 per cent. solution of shellac in alcohol in the cold with five parts of the same strength solution of borax in distilled water. The solutions should be mixed a drop at a time, and if a precipitate forms the mixture may be heated until clear. Enough methylen blue should be added to color it a deep blue. This makes one of the handiest inks for laboratory use, for with it notes can be made on glassware, slides, etc., which dry quickly and remain. Either a sharp pointed stick or a pen can be used to apply it. It is far handier than using gummed labels and writes better than the Faber pencil made for the same purpose. It will wash off afterwards.

Detroit.

WM. R. HUBBERT.

**LABORATORY PHOTOGRAPHY.***Devoted to Methods and Apparatus for Converting an Object into an Illustration.***PHOTOMICROGRAPHY WITH SIMPLE APPARATUS.**

In most of the work published on photomicrography great stress is laid on the apparatus, as if this constituted the main factor, the apparatus described being usually the costliest sort, and entirely beyond the means of the large number of persons who would be most helped by the use of photomicrographs. Dr. D. W. Dennis (Proceedings Indiana Academy of Science, 1900, p. 43) even going so far as to say that cheap apparatus is a delusion except for low power work of the simplest character and second grade quality. If this were so, and if the very costly apparatus which he and some others describe were necessary, there would be very little work done in photomicrography, and some of the best pictures would never have been made. In reality very good results can, and have been obtained with comparatively cheap apparatus, and without a waste of either time or material.

The factors, which seem to be the principal ones in the making of good photomicrographs, are a fairly good microscope, a steady camera stand, and a knowledge of the idiosyncrasies of the sections or mounts as to thickness, contrast of parts, color, and modifications of the light.

The apparatus which I use and find very serviceable is a "home-made" device, and consists of a piece of board about an inch thick, forty inches long, and about twelve inches wide, to which are attached a shelf to hold the microscope, and a sliding piece to carry the box or bellows of an ordinary camera. Under the shelf another piece of board is fastened to the first at right angles, and this assists in supporting the shelf, and also serves as a leg to help keep the apparatus in an upright position.

The back, leg, shelf, and sliding piece may be constructed from a piece of smooth pine board; the bolts and nuts used with the sliding piece are ordinary machine ones, that may be gotten at a hardware store. One of the bolts must have the same pitch as the hole in the camera box, by which it is fastened to the tripod.

The lens of the camera is removed, and a washer of felt is glued to the edge of the collar, so as to make a light tight connection with the eye-piece of the microscope. A slit is made in the side of the collar, and through this slit is fitted an elliptic-shaped piece of metal, having a round opening in one side, the other side being

**Fig. 1.**

A photomicrographic apparatus.

left entire, and also having a piece of the metal projecting on one side of the ellipse, to be used as a handle. The elliptic piece is the shutter for admitting or cutting off the light, and is manipulated by the projecting handle.

With the upright stand temporary mounts in water or other fluid media can be photographed. This is particularly valuable where research work is being done; as, for instance, organisms growing in moist chambers can be photographed in their various stages of development without any disturbance of the mount.

A good picture of the ordinary, stained permanent mount is easily enough obtained, but where the live tissue is used, and the fine detail is sought, greater care and skill are required. This is particularly true of the low organisms, where the fixing and

Fig. 2.—Transverse section of wood of *Pinus taeda*, taken with two-thirds apochromatic objective and No. 4 compensating ocular, x80.

staining cause a certain amount of contraction, and consequently a loss of the detail, and where one should necessarily use the live material to get a true picture. For work on mounts which show little contrast of parts the substage condenser provided with an upper and a lower diaphragm is invaluable. By means of these all the finer details of structure can be brought out, even in the colorless organisms.

A procedure which helps very much in the success of the picture is to expose for about four-fifths of the time required with both diaphragms almost closed, so as to get the least possible light, but the greatest contrast of the parts, then for the final one-fifth of the time, opening the diaphragms so as to admit a flood of light. In this way both detail and contrast are obtained.

For delicate focussing an engraver's glass is the handiest, as it can be set over the clear spot—made by a cover-glass

Fig. 3.—*Aspergillus oryzae*, moist chamber culture, taken with one-sixth inch objective and No. 1 eye-piece, x550.

attached to the ground glass of the camera by Canada balsam—and leaves the operator's hands free for manipulating the other parts.

The apochromatic lenses give much better results than can be obtained with the ordinary, so that it is well worth the while, if one does very much work, to pay the extra price for these lenses. In this connection also, a mechanical stage fitted to the microscope pays for itself in a short time in the rapidity and ease of finding a particular part of the mount.

Purdue University.

KATHERINE E. GOLDEN.

### A Photographic Apparatus for Pathological and Bacteriological Specimens.

Messrs. Folmer & Schwing of New York have lately constructed for Cornell Medical College a photographic apparatus for taking gross pathological or bacteriological specimens, which seems to be of a sufficiently serviceable nature to be put on record in the JOURNAL.

The main feature of the apparatus is that the camera can be used horizontally, vertically, or at any angle desired.

Fig. 1.—The apparatus in horizontal position.

The table on which the camera rests is solidly built of hard wood, 6 ft. 8 in. long, 1 ft. 8 in. wide, and 3 ft. 2 in. high.

On the table is a platform which carries the bed on which the copying camera runs. The platform being hinged on the table in front, can be raised from the horizontal to the vertical position, and is held in the latter, or at any intermediate angle, by slotted side arms, one on either side of the table, which can be securely locked by a bolt passing through the table, a milled head nut being attached at either end of the bolt.

The detachable adjustable specimen holder, 14½ inches square, is fastened by thumb screws to a telescopic bed, which may be extended 4 ft. 6 in. beyond

Fig. 2.—The apparatus in vertical position.

the front of the table, and can be locked at any point by means of two milled head clamping bolts.

The bed of the camera runs between two grooved side guides, and, like the telescopic bed, can be fastened at any point, a series of milled head binding

Fig. 3.—Small Pox skin. Natural size. Bausch & Lomb plastigmat.  
Camera in vertical position.

screws being provided for this purpose. The camera itself is of the ordinary "enlarging, copying, and reducing" design.

The adjustable front or specimen holder has a side shift as well as a rising and falling movement, and is fitted with a series of nested kits from lantern slides up to  $8 \times 10$ ; the kits being fitted with spring fingers, so that any transparent material, such as ground or opal glass, can be used for mounting a subject to be photographed by transmitted light.

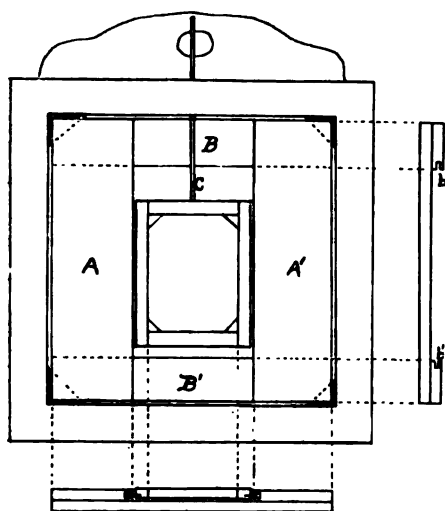
If photographs by transmitted light are to be taken, and it is found desirable to have the specimen tilted up against the window, the camera and telescopic front can be reversed on the tilting platform, and the camera elevated to any desired angle up to 90 degrees.

Cornell Medical College.

B. H. BUXTON.

### An Improvised Exposure-Test Kit.

Having no exposure-test kit with our microphotographic apparatus, we recently improvised a form which has served us so well that we feel that a few words concerning it may possibly be of value to some other worker.



The original kit furnished with the plateholder was about  $\frac{5}{16}$  inch thick. A kit frame just fitting into the plateholder was made by gluing two thin boards, A and A' (about  $\frac{5}{8}$  inch thick), with grooves cut in their inner edges, crosswise to two similar pieces, B and B'. The plate carrier was made from strips about  $\frac{3}{8}$  inch wide, and large enough to receive a  $4 \times 5$  plate. Thin triangular rests for the plate were glued in each corner. Small pieces of sheet brass driven into the sides of the carrier and moving freely in the grooves in A and A' serve as guides and allow the carrier a vertical movement of about four inches.

A brass rod, C, screwed into the top of the carrier and extending through a hole in the top of the plateholder, furnishes a means for adjusting the carrier. A set of interchangeable cardboard screens having horizontal slits of proper width are made to fit into the grooves b b' in B and B'. A set giving, for a single plate, either 3, 5, 9, or 17 exposures respectively has been found convenient. A scale marked on C gives the proper distances of movement.

If it is desired at any time to use the plateholder with the ordinary kit, the test kit can be easily sprung out at the bottom and removed. We found cigar box wood admirably well adapted for making the kit, since it is well seasoned and of the proper thickness.

BURTON J. HOWARD.

U. S. Dept. of Agri., Bureau of Chem.

### Note on Photographing Fruits.

Having occasion to make some photographs of apple to show the location of starch at certain stages of ripening of the fruit, the cut surface was covered with iodine solution by allowing to stand in a watch-glass of iodine in potassium iodid

solution. As some little time was required for the penetration of the iodine solution, there occurred considerable coloring brown in the non-starchy portion, which was not improved by allowing the iodine solution to evaporate on exposure to the air since then the counter staining by oxidation set in.

a.

Contrastive effects, however, were obtained by covering the freshly cut specimen with a bell-jar underneath which was burning a pinch of sulphur. It was

Photograph of apple showing (a) location of starch in bruised spots.

left for sixty to ninety seconds (a longer exposure interferes with the subsequent iodine staining), rinsed thoroughly in water, dipped under iodine solution till a good stain has been obtained, washed and exposed to the air thirty minutes or until the brown color due to the iodine solution has disappeared. In this manner good specimens for photographic purposes were obtained, in which the non-starchy portions were nearly white. The accompanying print shows the retention of starch in bruised parts, while in the normal portion the last traces are located in the peripheral region and adjacent to the fibrovascular bundles.

U. S. Dept. of Agri., Bureau of Chem.

BURTON J. HOWARD.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### III. PNEUMOCOCCUS, Croupous Pneumonia.

As a safeguard to the diagnosis a second preparation should be stained by Gram's method, as follows: Stain twenty minutes with gentian violet and transfer without washing to the decolorizing mixture:

Iodine, - - - - -	1 gram.
Potassium iodide, - - - - -	2 grams.
Distilled water, - - - - -	300 c.c.

which should act for about fifteen minutes; rinse thoroughly in alcohol. If any violet color remains decolorize again, rinse in alcohol, dry, and mount in balsam as described above.

The diplococci of pneumonia will appear well stained, with the capsule also stained. Most other bacteria will be decolorized.

FIG. V. *Pneumococcus* from the blood. Case fatal. Acetic acid method of staining. Magnified 1200 diameters.  $\frac{1}{2}$  oil immersion objective, Zeiss projection ocular No. 4.

FIG. VI. *Bacillus typhosis*, culture. Methyl-violet stain. Magnified 1200 diameters;  $\frac{1}{2}$  oil immersion objective, Zeiss projection ocular No. 4.

#### BACILLUS TYPHOSIS.

The determination of typhoid bacilli by direct microscopical examination of the faeces is not practical. Morphologically, they are so similar to the *Bacillus coli communis* that special bacteriological methods are necessary. Simple methods only are given here, which if carefully followed will yield satisfactory results. For more complete information the reader is referred to standard works on bacteriology.

Widal's reaction with dried blood offers the most practical assistance. The finger or lobe of the ear is carefully cleaned and dried. A large drop of blood from a needle prick should be collected on a clean and sterilized cover-glass or piece of mica. Either may be cleaned by washing in soap and water, thoroughly rinsed in clear water, and sterilized by passing through the flame of an alcohol or Bunsen burner several times with first one side and then the other uppermost. The drop of blood should be allowed to dry without heat, and then sent to the state health laboratory, or to the laboratory of some bacteriologist, for the completion of the test, as follows: With the platinum loop, a small drop of sterile bouillon is gently mixed with the dried blood until a dark brown fluid results. This is mixed on a cover-glass with a drop of pure bouillon culture of typhoid fever bacilli, and inverted over the concavity of a hollow ground slip. A little vaseline or oil is smeared around the edge of the cover-glass to prevent evaporation. It is now ready for examination under the microscope. At first the bacilli will be found actively motile, but if the blood is from a typhoid fever patient the motility will become gradually lessened and will almost entirely cease; at the same time the bacilli will become agglutinated in clumps. The reaction, if positive, should occur within half an hour.

It is hardly practical for the busy practitioner to have a suitable culture of typhoid bacilli ready for this test, but the establishment of state and municipal health laboratories in nearly every large city and state in the Union renders it

entirely practical to collect the blood, send it to the laboratory, and have a report on the following day.

#### THE EXAMINATION OF WATER FOR TYPHOID FEVER BACILLI.

Typhoid fever is considered an infectious disease, the source of infection being probably almost entirely in drinking water. It is necessary, therefore, that a bacteriological examination of the drinking water should be made when the disease is prevalent. The following preliminary preparation is necessary:

#### TO STERILIZE GLASS WARE.

Wash thoroughly one 500 c.c. Erlenmeyer flask, twenty-four test tubes, size 15 x 150 millimeters. Drain out the water and plug with cotton. Wash six 100 c.c. glass stoppered bottles, six 1 c.c. pipettes, wrap the bottles and pipettes each one separately in plain white paper and tie with a string. Place all in a hot air sterilizer. Gradually heat the sterilizer up to 150°C. for an hour or longer, until the cotton plugs and the paper are slightly charred. Allow the glassware to cool in the sterilizer, after which remove and store it in a drawer till needed.



Erlenmeyer Flask.



Graduated Pipette.

#### Physician's Hot Air Sterilizer.

Prepare nutrient bouillon as follows:

Distilled water,	- - - - -	500 c. c.
Extract of meat (Liebig's),	- - - - -	2.5 grams.
Dry peptone (Wittes),	- - - - -	15 grams.
Sodium chloride (common salt),	- - - - -	2.5 grams.
Grape sugar (or common sugar),	- - - - -	2.5 grams.

Mix and neutralize with a saturated solution of sodium carbonate, added drop by drop to the bouillon until it gives a very slight tinge of blue to neutral or red litmus paper. The bouillon should be boiled for fifteen or twenty minutes and filtered. If not clear, it is either too alkaline or there is a precipitate of albuminates. Test again for alkalinity, adding a little dilute acetic acid if

over alkaline. But the bouillon must remain neutral or slightly alkaline. If turbidity still exists, cool and add the white of an egg. Beat this in thoroughly, boil, and filter into a sterilized Erlenmeyer flask. Pour into each one of the test tubes about 10 c.c. of the bouillon, return the cotton plugs, and stand the tubes in a test-tube basket. When all are charged put the basket in a steam sterilizer and sterilize at the boiling point for twenty minutes on three consecutive days. When cool the tubes are ready for use, and will keep with occasional sterilization for months.

Prepare the following acid solution :

Carbolic acid crystals,	-	-	-	5 grams.
Hydrochloric acid, C. P.	-	-	-	4 grams.
Distilled water,	-	-	-	100 c. c.

#### Physician's Steam Sterilizer.

#### Physician's Incubator.

Add one, two, and three drops of this acid solution, respectively, to three of the test tubes of sterilized bouillon.

Collect the water to be examined in one of the sterile bottles, and as soon as possible put 1 c.c. of the water into each of the three prepared test tubes of bouillon, using one of the sterilized pipettes for measuring. Place the tubes in an incubator at 37° to 38°C. for thirty-six hours. If the bouillon becomes clouded, it will be due to the growth of the typhoid or colon bacillus, and the water should be classed as dangerous and unusable unless it is thoroughly boiled.

#### BACILLUS COLI COMMUNIS.

This bacillus is a normal inhabitant of the intestinal tract, and is of great interest, as it resembles morphologically the bacillus of typhoid fever. It requires a series of complicated bacteriological procedures to differentiate it from the typhoid bacillus. In water examinations it gives the same reactions

as the typhoid bacillus, but it is safe to say that if either is present in drinking water it should be boiled before using.

FIG. VII. *Bacillus coli* communis, culture. Stained with Loeffler's Alkaline Blue. Magnified 1200 diameters, Bausch & Lomb  $\frac{1}{4}$  oil immersion objective. Compensating Photo ocular No. 2.

FIG. VIII. Gonococcus. Cover slip prepared direct from the pus. Methyl violet stain. Magnified 1400 diameters, Bausch & Lomb  $\frac{1}{4}$  oil immersion objective. Compensating Photo ocular.

#### GONOCOCCUS.

The preparation of mounted slides of this bacterium is easy and simple. To examine Gonococcus spread a thin layer of the fresh pus on a clean cover-glass, held in a Cornet forceps, using the inoculating needle. Pass the cover through the flame three times, film side up, and use any of the anilin stains according to formulæ given. Beautiful crisp staining may be obtained with methyl violet, with the advantage that if the preparation is over-stained any amount may be washed out with alcohol and its action immediately stopped by applying water. The bacteria will be found in pairs, located in or on the pus cells. In appearance they are like two biscuits, with the flat sides toward each other, and separated by a minute distance usually equal to about half the diameter of the bacteria.

WILLIAM H. KNAP.

Harvey Medical College.

### The Technique of Biological Projection and Anesthesia of Animals.

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Biological projection, as used in this series of articles, includes all the work with stereopticons, projection microscopes, and their modifications, using solar, electric, or oxyhydrogen lights, by which live plants and animals are exhibited as living charts on a screen. The illustration gives an example of the work done with lenses of low or medium power. The objective used was a Leitz No. 3, distance from projection microscope to screen thirty feet, magnification 650 diameters. The study of small live animals and plants under the hand lens, or compound microscope, is incomparably more valuable than the study, even for greater lengths of time, of the best pictures and diagrams, useful as these are in their place. Wall charts, lantern slides, models, and other devices have filled a

useful place in teaching botany and zoölogy, limited as their value has been. One needs only to see upon the screen the magnified pictures of live plants and animals exhibiting their motions, morphology, much of their anatomy, and even physiological phenomena, with all the parts in normal colors, sizes, and relations, to appreciate the value of the new projection methods which produce instructive, interesting and valuable living charts.

The apparatus, methods, and especially the little kinks in manipulation of the apparatus and living organisms, on which much of the success in biological projection depends, will be the subject matter of this series of articles. The author makes no claims to have solved all the problems or that other workers in this line have not invented methods which he will be pleased to adopt; but he does hope to assist many busy workers in the attainment of long wished for results and to bring biological projection into the place which it alone can fill.

To accomplish certain and definite results when using many species of active animals, it is necessary to reduce or entirely check the voluntary activities of the animal by the use of suitable anesthetics or hypnotics. While looking for a hypnotic applicable to various animal types, the writer came across a new hypnotic used by the medical profession and called Chloretone by its manufacturers.\* Beginning its use in the spring of 1900 and finding it of exceptional value, exact experimental tests were made and formulæ for its use worked out on typical animals ranging from amœbæ to warm blooded vertebrates. The methods were demonstrated to the members of the Biological Round Table (teachers of biology in the Chicago high schools) and in the summer of 1901 were a part of a course in the "Technique of Biological Projection and Hypnosis of Animals" in the University of Chicago. The term anesthesia is now used instead of hypnosis, the latter term carrying the idea to many people that mental processes, rather than chemical agents, are used in inducing the passive state in animals used in this work. As these methods of producing anesthesia very greatly increase the number of species available for projection, they necessarily become a part of the treatment of the subject.

A general idea of the possibilities of biological projection may be gained from an enumeration of some of the common plants and animals which can be made to exhibit not only their external forms and motions, if they are motil, or like *Spongilla* and fresh-water clams produce currents in the water, but are, or can be made, sufficiently transparent to permit of entire or partial optical sections being projected with sufficient magnification and illumination to make the living chart visible to an entire class or an audience of several hundred people. The types enumerated below are a part of those regularly used in class studies and public lectures.

Beginning with the bacteria, it is possible to exhibit medium sized motil species when using sunlight, and large species, e. g., *Spirillum volutans*, with electric arc illuminant and ordinary microscope objectives. Yeasts, pleurococcus, diatoms and desmids present a wide range of simple types, many of them available for high power work up to 10,000 diameters. *Volvox* makes a most interesting picture, combining the various stages, sizes, graceful motions and details of struc-

\* Parke, Davis & Co., Detroit, Mich.

ture. The common pond scum, *Spirogyra*, is an excellent type with which to practice medium and high power projection. The vegetative stage affords fine examples of cell division, many species exhibit the nuclei clearly, and the reproductive stages are brought out with great clearness, even to the structure of the zygospores. *Vaucheria* is an easy and instructive object. *Chara* or *Nitella* affords opportunity to use any one or all of a series of lenses, beginning with a quarter-size projector, such as is in common use for lantern slides, which shows a section of the plant three inches or more long and magnified about forty dia-

Fig. 1. Hydra (x 650). class making drawing. Illumination of screen by porte-lumiere, of room from window. One exposure.

meters at thirty feet, then low power objectives for gross structure, higher power for young or mature antheridia and oogonia, and high power objectives for spermatic filaments and the circulation of protoplasm.

Marchantias and mosses, the prothallia of ferns and the same with a young sporophyte attached, embryo plants, and parts or sections of large specimens present a wide range of types. The physiological action of green plants under the stimulus of sunlight can be as perfectly shown with the electric arc light as

with solar apparatus. An interesting series of experiments exhibits (1) the evolution of bubbles of oxygen during starch formation, (2) the effects of heat and slight or severe cold on the physiological activity of plant protoplasm, (3) the iodine test for starch in a plant taken in full vigor of growth, (4) the swelling of starch grains when cooked and their change to a translucent condition, and (5) if common potato is used in the previous experiment, the cellulose walls of the cells which contained the starch grains.

Turning now to animal types, *amæbae* give living charts which exhibit all details of structure and motion. Many of the *infusoria*, both free swimming and colonial forms, exhibit their most interesting peculiarities and are easily manipulated. The structure of *Spongilla*, the currents of water from the oscula, and the contractility of the cells of sponge flesh surrounding the oscula are readily shown with low power objectives. *Hydras* are never failing objects of interest and instruction in their remarkable variations of shape and motion, their habits of feeding, their modes of reproduction, including all the stages of budding and the detachment of the mature bud, and the swarming spermatozoa in the spermary. Many species of worms are excellent objects, small earthworms especially so. All the coarser anatomical details and the nephridia are visible, the movements of the gizzard and its contents, the peristaltic action of the intestine, and the pulsation of the dorsal vessel and the so-called "hearts." The *Arthropoda* afford an endless amount of available material including adults and all stages of metamorphosis, which are easily shown by a series including mosquito's eggs, small and large larvæ, pupæ, and imago. Respiratory apparatus of various types, the circulation of the blood, the pulsation of the heart, even its valvular action, and the ventral nerve cords and ganglia are among the possibilities in projecting the arthropods of various common species. Small univalve or bivalve mollusks display their characteristic modes of locomotion and the action of the siphons. Large fresh-water clams, when properly prepared, afford a striking exhibition of the heart's systolic and diastolic action and of the instant response of the heart to changed conditions of heat or cold. Among the vertebrates, small fish and tadpoles serve for demonstrations of swimming, breathing, feeding and the circulation of the blood. Small tree-frogs show their adaptation to their habitat. Live chick embryos, during the earlier stages, exhibit various details, including the pulsation of the heart of a warm-blooded vertebrate.

Many additional experiments might be cited, which have utilized land and fresh-water species, and the adaptability of great numbers of marine species is evident at once. The wide range of organisms and life phenomena, from motile bacteria to the pulsating heart of a warm-blooded vertebrate, to which these methods are adapted, the definite results obtained, and the comparatively small cost of the necessary apparatus, commend living charts to all who are interested in bringing students and popular audiences into the closest possible contact with actual life phenomena as well as its structural forms.

A. H. COLE.

University of Chicago.

## A Review of the Existing Methods for Cultivating Anaerobic Bacteria.

Since the discovery by Pasteur in 1861 of the fact that some species of bacteria can thrive in the absence of oxygen only, various methods have been introduced involving many devices for the study of anærobic bacteria. Notwithstanding this fact, our knowledge of anærobes is as yet very limited. While bacteriology has made rapid progress along the line of ærobic species, a comparatively small number of anærobic species have been carefully studied and identified.

Concerning the character of some of the known anærobes, such as the bacilli of tetanus, of malignant œdema, and of black leg, it becomes evident that the cause of the slow development in the study of anærobic species does not lie in the fact that these species are of less pathologic and economic importance than their brothers the ærobes. Nor can it be attributed to the assumption that this class of organisms covers a very limited number of species only, for the results of bacteriological research of recent years are rapidly revealing the existence of a liberal distribution of anærobic bacteria in nature.

On the other hand, the bacteriologist realizes that the production of anærobic conditions for bacterial growth involves greater difficulties and meets more frequently with failure than the simple cultivation of ærobes. It is, therefore, reasonable to recognize this greater difficulty, complexity and expense of technique as the most potent obstacle in anærobic research.

While, from the above statement, it is obvious that none of the methods for anærobic cultures are quite so simple and easy as those for ærobes, still, a thorough review of the multitude of methods introduced and devices invented shows that there are some that involve little difficulty, are simple, and can be manipulated in any laboratory. Methods for the cultivation of anærobic bacteria are referred to in most text-books and manuals on bacteriology; however, space in such books does not permit a full list of these methods, with directions for practical application; and a search through the entire bacteriological literature in pursuit of the easiest, simplest and most efficient method is a task that does not appeal to the investigator eager to grow cultures with the greatest economy of time. In order to fill this gap, to afford the investigator easy access to the existing methods, and, by so doing encouraging the study of anærobic bacteria, it was thought desirable to carefully review the literature on this subject, and to present the principal methods and their modifications in this article.

For the purpose of being able to treat the diverse methods in logical order, the principles upon which they are based are arranged in the following classification, in which order they will be reviewed.

The Anærobic conditions are brought about:

1. By formation of a vacuum.
2. By replacement of air by inert gases.

3. By absorption of oxygen.
4. By reduction of oxygen.
5. By exclusion of atmospheric oxygen by means of various physical principles and mechanical devices.
6. By the combined application of any two or more of the above principles.

#### METHODS FOR CULTIVATING ANAEROBIC BACTERIA IN A VACUUM.

In the search for methods that would enable the investigator to obtain cultures in the absence of oxygen, the principle of exhaustion was naturally one of the first resorted to. It was much used in early research concerning anærobic bacteria. While absolute anærobic conditions can be obtained in a vacuum only, the difficulty of successful evacuation and the fact that a good vacuum pump generally lies beyond the means of small laboratories, explain the reason why the principle of exhaustion has been losing ground in recent years and is gradually being replaced by more simple and less expensive means to produce anærobic conditions.

*Pasteur's Method.*—Use a small flask with a long neck or test tube; fill it one-half (in case of flask) or one-third (in case of test tube) with the inoculated medium. Constrict the neck at *b* (Fig. 1) and connect the end of the neck *c* with the vacuum pump. While evacuating place the bulb of the flask or the tube in a water bath at 37° C., causing the liquid to boil. Evacuate for thirty minutes; then, while still exhausting, seal at *b* in the flame.



FIG. 1.

According to Fitz this is the oldest and simplest method; it was introduced by Pasteur and tested by Cohn, Lister, Tyndall, Aitken, and Fitz. Nenki introduced a method identical with the above.

*Roux*, in 1887, modified Pasteur's apparatus as shown in Fig. 2. This type of apparatus has been in constant use in Pasteur's laboratory for many years. The bulbs *a* and *a'* hold the medium, which is introduced through the lateral capillary tubes *b* and *b'* by means of suction at *c*.

*Method.*—Push a loose cotton plug into tube *c*. Seal the lateral tubes *b* and *b'* in the flame and sterilize the apparatus in the hot air sterilizer. Break off the tips on the lateral tubes *b* and *b'* and immerse the tubes in bouillon. Apply suction at *c*. When the tubes *a* and *a'* are about one-third full seal the ends of the lateral tubes again and sterilize in steam sterilizer. When cool reopen the lateral tubes and introduce the inoculating material by suction at *c*. Melt off the lateral tubes in the flame and connect *c* with a vacuum pump. When completely evacuated seal tube *c* at its constriction and remove the apparatus to the incubator.

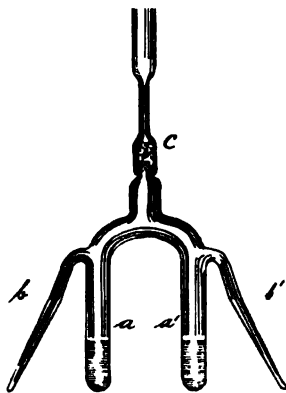


FIG. 2.

In this apparatus two cultures may be grown simultaneously. Apparatus shown in Fig. 3 works on the same principle with the exception that it can take care of only one culture at a time. The latter can also be used for cultures on solid media and Esmarch roll cultures.

*Gruber's Method.*—Take a long test tube 22 to 25 cm. long. About 15 cm. from the bottom draw it out into a constriction as illustrated in Fig. 4, plug the tube with cotton as usual, and sterilize. By means of a funnel pour about 10 c. c. of the sterile medium and 2 c. c. of sterile water into the tube and sterilize. Then inoculate the medium as usual. Push the cotton plug well

down into the tube and insert the rubber stopper. Connect the glass tube in the rubber stopper with a vacuum pump and immerse the lower part of the test tube in water at 37° C. in case of bouillon or gelatin, and at 42° C. in case of agar. In ten to fifteen minutes the tube is evacuated. To avoid wetting of the cotton plug by the boiling and foaming medium, the constriction may be slightly and carefully flamed. While still evacuating, the tube is sealed in the flame. If agar is used, cool the hermetically sealed tube in a water bath to 40° C., then roll it until the medium congeals; in case of gelatin cool slowly in the air by constant rolling of the tube in the hand, so that at room temperature the vacuum is filled with vapor. In case of bouillon cultures the operation is finished as soon as the tube is sealed.

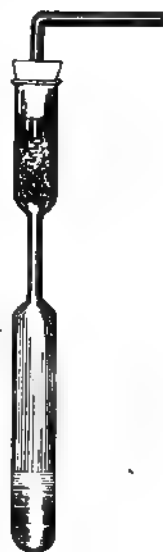


FIG. 4.

*Roux's tube* for potato cultures in vacuum is operated in the same way, but the potato is introduced into the test tube and the inoculation is made before the tube is constricted. The same investigator also recommended for potato cultures a tube as shown in Fig. 5. As soon as the potato in the culture is inoculated the tube is sealed at *a*, and the lateral tube closed with a cotton plug is connected with the vacuum pump. When evacuated the lateral tube is sealed in the flame at *c*.

FIG. 5.

*Novy* constructed an apparatus for plate cultures in vacuum (Fig. 6). It consists of a cylinder ending in a firm, broad rim. On the rim is placed a thick rubber ring. The cylinder is closed by a bell jar, the lower rim of which corresponds with the upper rim of the cylinder. The whole apparatus terminates in a stop-cock.

*Method.*—Place the inoculated Petri dishes in the

FIG. 6.

cylinder, invert the bell jar over it, and close firmly by applying clamps with rubber lined jaws to the union of the cylinder and bell jar. One end of the turn cock ( $x-y$ ) is connected with a thick-walled rubber tubing which is closed by a screw compressor. The other end is connected with a vacuum pump and when evacuation is complete the turn cock is turned and the vacuum pump is disconnected.

*Zupinski* in 1898 introduced a new and simple method of evacuation. It is based on the principle of Toricelli's vacuum. The apparatus (Fig. 7) consists of a tube which is constricted at each end, each constriction carrying a glass turn cock, a long glass tube holding a column of mercury, and a small beaker filled with mercury.

*Method.*—Fill tube  $m$  completely with medium; inoculate through  $c$ , connect the constriction at  $c'$  by means of a piece of stout, elastic rubber tubing with glass rod  $g$ . Close both turn cocks. Reverse the whole apparatus, fill the glass tube  $g$  with mercury; close the end with the finger and return the apparatus to its original position, Fig. 7, standing the open end of the glass tube  $g$  in a beaker  $b$  containing mercury. The column of mercury falls to 750 mm., and above it there is an absolute vacuum, the Toricellian vacuum. Now open cock  $c'$ , instantly the medium descends and above it there is formed an absolute vacuum. Close cock  $c'$  and paraffin it. Remove glass tube  $g$  and put the apparatus into the incubator. The diameter and length of glass tube  $g$  govern the capacity of the vacuum. The gases produced by bacterial activity can evolve without endangering the apparatus and can be examined without interference by other gases.

For the above method any flask or test tube may be used. When filled with inoculated medium it is closed with an air tight, mono-perforated rubber stopper. The perforation carries a snugly fitting glass tube which is connected with a long, heavy glass tube by means of a short piece of rubber tubing, carrying a firm clamp, which serves in the place of the glass turn cock. The manipulation is the same as with the previous apparatus.

While the few devices and methods described in this category appear to be the only ones specially designed for vacuum cultures, there exist a large number of apparatus which may be and have been used for this purpose. Thus most of the devices invented for anærobic cultures in an atmosphere of inert gases may easily be adapted for vacuum cultures. For their description the reader is referred to their respective classes.

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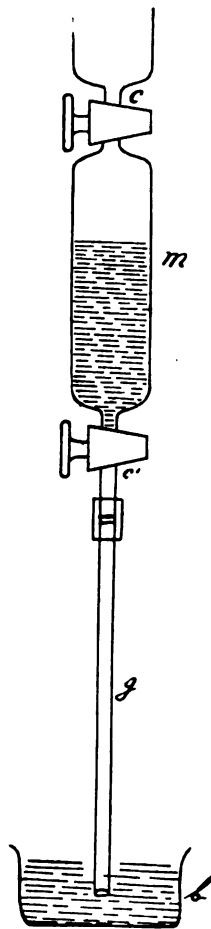


FIG. 7.

1. I wish to express my appreciation to Dr. V. A. Moore and Dr. E. M. Chamot for valuable suggestions in this work.

2. The entire bibliography will appear at the end of this series.

## LABORATORY OUTLINES.

For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### SERIES I—THALLOPHYTA. A STUDY OF THE LOWEST, NON-SEXUAL FORMS.

#### IV. *Pleurococcus vulgaris* (Menegh.). Order, Pleurococcales. Family, Pleurococcaceæ.

This is a unicellular green alga which is very commonly found forming a green, powdery layer on the bark on the north side of trees, on wooden fences, etc.

1. Scrape off some of the green powder from a piece of moist bark and mount in water. Draw a single plant under high power, showing the thick cellulose wall and the chloroplasts.

2. Notice that the cells (individuals) have a tendency to hang together for some time after division. Study and draw aggregates or colonies of two, three, four, and eight cells still united. In how many directions do the cells divide? Describe the color, shape, and habitat of the plant. How does it get its food? Notice that it must be exposed to long periods of drouth.

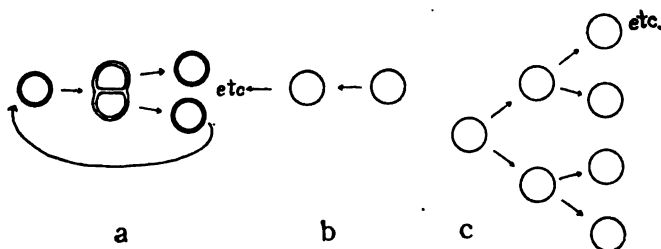


Fig. 2.—Life Cycle of *Pleurococcus*.

3. Its life cycle may be stated as follows: increase in size of the cell, division of the cell into two, separation of the daughter cells. Taking no account of the fact that the cells hang together for some time after division, make a diagram illustrating this as indicated in Fig. 2, a.

4. Make a diagram showing the ancestors of one individual for ten generations. See Fig. 2, b.

5. Make a diagram showing the descendants of one individual for ten generations. See Fig. 2, c.

6. All plants and animals, whether high or low, are single cells in the first stage of their life. Therefore, in the higher forms, the egg or spore also passes through the two, four, etc., celled stages, and in these first stages the cells may also represent a loose aggregate or colony, since in many cases, if the cells are separated from each other by artificial means, two or more plants or animals may be obtained from the egg, which would otherwise have produced only one individual.

*Pleurococcus* shows how it is possible for a plant to pass from a unicellular

condition to a colony, and from the condition of a colony to a multicellular plant. By what means could this be accomplished? The mechanical reason for the division of one of these cells may be dependent on the following facts: All food and waste material must pass through the wall. Now the surface of a sphere is equal to  $\pi D^2$  and the volume is equal to  $\frac{1}{6} \pi D^3$ , therefore, as the sphere increases in size, the surface continues to become less in proportion to the volume. How could a cell increase indefinitely in size and still keep the surface and volume in about the same ratio? What disadvantage or limit would there be to such a process? These plants have potential immortality, i. e., they do not grow old and die, except by accident. Natural death of an organism appears to be an acquired character. This plant, with a number of others to follow, is unicellular and without sexuality. It belongs to the lowest sub-kingdom of plants, usually called the *Protophyta*.

V. *Merismopedia* sp. Class, Cyanophyceæ. Order, Coccogonales. Family, Chroococcaceæ.

This organism can usually be found in the sediment of creeks, ponds, or lakes, especially in shady places where there is some decaying vegetable matter.

1. Mount some of the sediment and examine under high power. Look for minute, blue-green, more or less rectangular plates of cells. Find colonies of various sizes, select a perfect one and draw, showing the arrangement of the cells.

2. In how many directions does cell division take place? How does the colony break up into smaller pieces? Such a flat layer of cells is called a superficial aggregate. Neither plastids nor nuclei are visible in these cells. They may be absent entirely. The bluish color is due to the presence of a peculiar coloring matter in addition to the chlorophyll. Notice the gelatinous nature of the cell wall. Write a careful description of the plant.

VI. (a) *Lyngbya* sp. Class, Cyanophyceæ. Order, Pylonematales. Family, Oscillatoriaceæ.

The species known as *Lyngbya wollei* Farl., which produces large brownish-black masses in rivers and ponds, or any other large species, may be used. A large species, appearing like a brown or black slimy layer, quite common in greenhouses and other moist situations, is also very good for study. This form can be kept indefinitely in a moist jar of earth.

1. Mount a small mass of the slimy material in water, and study under low power. Draw several of the greenish-brown threads or filaments. Notice the disc-like cells which make up the filament. Describe the general character of these plants.

2. Under high power study a single filament. Draw part of a filament, showing the end cell. Why is the end cell more or less hemispherical and the others disc-shaped? Notice the dark granules. Where are they situated? Notice the thick sheath surrounding the cells. Draw a single cell.

3. Study and describe the slow, oscillating movement of the filaments. Also watch the movement of some short, free filaments.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

**Hegler, R.** Untersuchungen über die Organization der Phycocchromaceenzelle. *Jahrb. f. wiss. Bot.* 36: 229-354, pls. 5-6, 1901.

This work has been expected ever since 1895, but the poor health of the author delayed the investigation. After

the fatal illness the manuscript, quite ready for the press, was published by Prof. G. Karsten, who states that since the appearance of A. Fischer's work, the author most carefully re-examined the disputed points, but without deeming it necessary to change his views. The photomicrographs, with which the paper is illustrated, do but scant justice to the beautiful preparations which the reviewer had the privilege of examining. About forty pages are devoted to a critical review of the literature of the subject. The rest of the paper, about eighty pages, contains an account of Dr. Hegler's prolonged investigations. The principal conclusions are about as follows:

There are no naked protoplasts in the Cyanophyceæ, all cells being provided with cell membranes which in the heterocysts consist of cellulose and in other cells consist principally of chitin.

In the protoplast, or cell contents, may be distinguished an outer, color containing layer and an inner colorless portion. The coloring matter is in the form of extremely small granules so closely crowded as to give the impression of an homogeneous color. The chlorophyll and phycocyan are contained in the same granules and these granules are to be regarded as the chromatophores of the Cyanophyceæ. Starch or starch-like material is not present, but glycogen can be identified and is the first recognizable product of assimilation. Albuminoid crystals and slime vacuoles are never found in the colorless, central portion of the cell. The albuminoid crystals are particularly abundant in the heterocysts and spores, but are often entirely lacking in rapidly growing vegetative cells.

Whether a nucleus is present or not is the most important morphological question in connection with the Cyanophyceæ and Bacteria since these are the only organisms in which a nucleus has not been positively identified. The bearing of this question upon present morphological theories of heredity is evident. Since previous methods have failed to solve the problem, he devoted much attention to fixing and staining, and finally recommended the following:

Saturated aqueous solution of SO <sub>2</sub>	-	-	7 parts.
94 per cent. alcohol	-	-	93 parts.

Mix just before using and fix for twelve to twenty-four hours. Wash with alcohol.

Another fixing agent which gives good results is:

40 per cent. formalin	-	-	-	5 parts.
94 per cent. alcohol	-	-	-	95 parts.

Wash in fifty per cent. alcohol. The first named fixing agent, however, allows a sharper staining of the nuclear figures.

Fuchsin, safranin and gentian violet do not stain well; methyl green, iodine green and methyl blue are better. The following method gave the best results:

Crystals of ammonia alum	-	-	-	-	75 parts.
Water	-	-	-	-	750 parts.

Dissolve the crystals in the water and add

Glycerine	-	-	-	-	125 parts.
Alcohol (94 per cent.)	-	-	-	-	100 parts.
Saturated alcoholic solution of hæmatoxylin					25 parts.

The solution must stand for several weeks in a beaker, covered only by a piece of filter paper, before it is ready for use. Then stain for twenty-four hours in a mixture of 10 parts of the above solution in 100 parts of a one per cent. aqueous solution of formalin. Wash in running water at least one hour and then differentiate in

Saturated alcoholic solution of picric acid	-	1 part.
Water	-	1 part.
Alcohol (94 per cent.)	-	2 parts.

The differentiation will generally require only a few seconds. Rinse in 75 per cent. alcohol and examine; if still too deeply stained, transfer again to the differentiating solution, and so continue until the stain is clear and sharp, then wash in running water for an hour, transfer to 50, 75, 94 per cent. and absolute alcohol, clear in toluol, and mount in dammar.

From such preparations the following conclusions are drawn: The "Centralkörper," or central body of the Cyanophyceæ is a nucleus. The most important reason for this conclusion is the behavior of the central body during cell division. In all cells of the Cyanophyceæ, except the heterocysts, the nuclei of which degenerate very early, a single nucleus is present, its form depending largely upon the shape of the cell. The resting nucleus consists of a slightly staining ground mass in which are embedded numerous deeply staining granules. These granules from their behavior during division, their reaction to stains and digestive fluids, are to be identified with the chromatin granules of higher plants. They are not the "red granules" of Bütschli. The nucleus differs from that of higher plants in not possessing a nucleolus or nuclear membrane. During nuclear division the granules fuse into chromosomes which separate as division proceeds. Strands which represent spindle fibers may be seen between the separating groups. The wall first appears as a ring midway between the daughter nuclei and gradually grows toward the center until the partition is complete.

The writer believes that he has shown positively that the "Centralkörper" is to be identified with the nucleus of higher plants.

It is interesting to note that Zacharias, in a lengthy and—in our opinion—somewhat prejudiced review of Dr. Hegler's paper (Bot. Zeit. 59: 322–327, 1901) disputes the accuracy of these conclusions and insists that there is no nucleus in the Cyanophyceæ.

C. J. C.

Stevens, F. L. Gametogenesis and Fertilization in *Albugo*. Contributions from the Hull Botanical Laboratory XXIX. Botanical Gazette, 32: 77-98, 157-169, 238-261, pls. 1-4, 1901.

The striking differences presented by the conditions in *Albugo Bliti* and *A. candida* induced Prof. Stevens to make a comparative study of the genus. All

material was fixed in chromo-acetic acid and stained in Flemming's safranin-gentian violet-orange combination. *Albugo Portulacæ* and *A. Tragopogonis* were thoroughly studied, and *A. candida* was again re-examined. The principal results are as follows:

In *A. Portulacæ* and in *A. Tragopogonis* a multinucleate oosphere develops as in *A. Bliti*. In both forms two mitoses occur in oogenesis and in spermatogenesis, the nuclear figure of the second mitosis being clearly distinguished from that of the first by its diminished kinoplasm. In *A. Portulacæ* the antheridial tube is multinucleate and the numerous antheridial nuclei fuse in pairs with the numerous egg nuclei. In *A. Tragopogonis* the multinucleate oosphere becomes reduced to the uninucleate condition by the disorganization of the supernumerary nuclei, after which the antheridial tube conveys one or more nuclei into the oosphere, where one antheridial nucleus fuses with the egg nucleus. In *A. Portulacæ* the fusion nuclei pass the winter without further change, while in *A. Tragopogonis* the fusion nucleus divides repeatedly, consequently giving rise to a multinucleate winter oospore. The supernumerary nuclei are phylogenetically gametes, which degenerate as in the *Fucacæ* and some other forms. The four species, *A. Portulacæ*, *A. Bliti*, *A. Tragopogonis* and *A. candida* constitute a series in which the cœnocentrum increases in complexity, the receptive papilla decreases, and the number of functional nuclei decreases. Of these *A. Portulacæ* is probably the most primitive form. The relation between *Albugo*, *Peronospora* and *Saprolegnia* is emphasized by their cytology and all are probably derived from a common ancestor having a multinucleate oosphere. If the Phycomycetes are related to *Vaucheria* it is from a period before the attainment of the uninucleate oosphere by *Vaucheria*.

C. J. C.

Hinze, G. Ueber den Bau der Zellen von *Beggiatoa mirabilis* Cohn. Ber. d. deutsch. bot. Gesell. 19: 369-373, pl. 18, 1901.

As far as possible, the cells were studied in the living condition, but for the finer details microtome sections were

made from material fixed in Flemming's solution and stained in Heidenhain's iron-alum-hæmatoxylin. The cells are surrounded by a double wall. The protoplasm, as in the higher plants, forms a layer inside the membrane and contains vacuoles filled with cell sap. No differentiation into an outer protoplasmic layer and a central body could be demonstrated. There is no nucleus. The transverse walls give no cellulose reaction, but, on the contrary, stain with ruthenium red, safranin and methyl blue. It is worthy of note that the cells are not plasmolyzed by solutions of saltpeter, sugar, and glycerine. When a contraction of the plasma body is produced, either the entire membrane collapses with it or only the inner layer of the membrane remains in connection with the plasma body. After the sulphur has been dissolved out with absolute alcohol, there still remain bodies which stain with hæmatoxylin. These the writer regards as chromatin granules. Besides the chromatin granules, there is also a substance which is apparently a carbohydrate nearly related to starch.

In cell division the transverse wall first appears as a ring on the inner surface of the cell membrane. This then grows toward the center of the cell as in *Spirogyra*. The formation of the transverse walls can be observed in living cells, but preparations deeply stained with hæmatoxylin are more favorable. C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

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Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Spuler, A.** Ueber eine neue Stueckfaerbe-  
methode. Deutsche med. Wochenschr. 27:  
116, 1901.

distilled water is added and the mixture filtered. Pieces of tissue are put into this solution for twenty-four hours or longer, in a warm place on the paraffin oven. They are then washed off and soaked for a long time in a bath of iron-alum, which quickly changes the color from red to black. After this treatment the preparations are thoroughly washed in distilled water and imbedded in the usual way. Iron-alum, tannin or any other mordant may be used simultaneously with the stain.

This new method gives homogeneously stained bulk material, preparations from which prove highly satisfactory for projection purposes; since they have an intense black color. The cell contours are as clear in outline as the nuclei, so that the preparation gives the impression of a pen drawing. A. M. C.

**Willebrand, E. A. V.** Eine Methode fur gleich-  
zeitige Combinationsfaerbung von Blutt-  
rocken praeparaten mit Eosin und Methylen-  
blau. Deutsche med. Wochenschr. 27: 59,  
1901.

containing methylen blue in excess, which gave most satisfactory results; five per cent. eosin was dissolved in seventy per cent. alcohol, to which was added equal parts of concentrated aqueous solution of methylen blue. Used in this form the stain gives preparations colored a diffuse blue. Adding about ten to fifteen drops of a one per cent. solution of acetic acid to about 50 c. c. of the stain, a very different result is obtained. If the blood preparations are well fixed in dry heat, absolute alcohol, or one per cent. formol-alcohol, and stained for five to ten minutes with repeated warming and subsequent washing, with no decolorization, the following result is obtained: Erythrocytes are red, nuclei dark blue, neutrophile granules violet, acidophile red, and the granules of the mast cells are intense blue. A. M. C.

**Heinz, R.** Ueber Blutdegeneration und Regen-  
eration. Beitr. z. pathol. Anat. u. z. allgem.  
Pathol. 29: 299-404, 3 Tfn., 1901.

Bone marrow concerned in regenera-  
tion shows a very complex structure.  
The significance of the single elements  
depends in large measure on the histological technique of the method used.  
Staining methods are extremely well worked out. There are in most cases sev-  
eral staining and fixing methods available. For example, there are many hema-

globin stains, yet none is specific. If a positive result is obtained by using orange-eosin, it is not certain that all the substance so stained is hemaglobin; moreover, a lack of blood material is not necessarily made evident by stains. The fixative used is an important factor in the preservation of hemaglobin. To study erythroblasts, the most essential elements in bone marrow, especially to study the nucleus and the hemaglobin contents of the protoplasm, the investigation must be made on marrow from recently killed animals. The marrow of the normal dog is grey-red, fatty, easily crumbling; shows microscopically very much fat. In regeneration stages it is compact, markedly harder, free from fat, and deep wine-red. Marrow was taken from the epiphysis of the femur and cylindrical pieces were cut from the split long bone; hence decalcification was avoided. For preserving blood degeneration and regeneration material these mixtures were used: formol-salt solution (ten to fifteen per cent. formol in five per cent. salt solution) and formol-sublimate-acetic salt solution. (Formol and sublimate three to five per cent., acetic acid 0.5 per cent.) Formol is invaluable for all work concerning hemaglobin since the formaldehyde makes with it a met-hemaglobin-like substance of great permanency. This substance has a darker color than hemaglobin itself, so that the red blood cells appear more distinctly marked than in fresh preparations. The second fixative is particularly good for degenerating finer structures, as granules and nuclear figures. The liquid is warmed to blood heat and kept in the oven at  $37.5^{\circ}$  until the fluid has penetrated to the middle, requiring two to three hours. Longer continuance will make the tissue brittle and stain badly. Then follows twenty-four hours' washing in running water, hardening in increasing strengths of alcohol from fifty per cent. upward. Removing of sublimate by iodine-alcohol and imbedding in chloroform-paraffin complete the steps. For the formol process ten per cent. is used for twenty-four hours, this gives the best results with granules, mitoses and plasma carrying hemaglobin. Staining followed in alum-carmin or alum-cochineal, hematoxylin or hematein (Mayer's hæmalum). A protoplasmic stain is necessary, and hæmalum-orange was found to be a good combination, since orange brings out clearly the principal blood elements. In demonstration of the finer structure Ehrlich-Heidenhain-Biondi's triple stain gave the best results.

A. M. C.

Herport, K. Die Reifung und Befruchtung des Eies von *Petromyzon fluviatilis*. Arch. f. mikros. Anat. 57: 54-95, 1900.

Von Rath's picro-osmic-platinic chloride-acetic mixture and picric-platinic chloride-acetic or aceto-sublimate were

used for fixation. The eggs were imbedded singly in paraffin to ensure their orientation. Heidenhain's iron-hematoxylin, with and without eosin, and Delafield's hematoxylin together with fuchsin and saffranin gave excellent results. Eggs preserved long in alcohol cut very badly and gave almost unusable preparations.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Sars, G. O. Contributions to the Knowledge of the Fresh Water Entomostraca of South America. I. Cladocera. Arch. f. Math., og. Naturvidenskab, 22: 1-102, 12 pls. II. Copepoda-Ostracoda. Ibid, 24: 1-52, 8 pls. 1901.

A large part of the material upon which these studies were made was secured from aquaria which had been stocked with dried mud and dried aquatic vegetation from Brazil and Argentina.

Aquaria thus stocked and provided with the proper amount of aquatic vegetation produced a succession of *Cladocera* throughout the season. Aquatic plants introduced into such aquaria should be carefully freed from all mud, and washed in filtered water to prevent contamination with indigenous species. Confinement in aquaria seemed not to interfere with the usual cycle of parthenogenetic and sexual generations; for example, the males of *Diaphanosoma*, *Simocephalus*, *Macrothrix*, *Leydigioopsis*, *Alonella*, *Chydorus*, and *Euryalona* were found in such aquaria. It is thus possible to secure in many cases the two sexes, and both parthenogenetic and ephippial ova, as well as the immature and adult stages. This method offers the further advantage of affording opportunity for the study of the habits of the living *Cladocera*. Dried mud from the bottom of aquaria may be kept for stocking new aquaria in subsequent years. Among the *Copepoda*, the *Centropagidae* alone can be reared by this method. The *Cyclopidae* and *Harpacticidae* seem not to form the resistant ova which survive the drying process. The method here described is a valuable one, not only for the study of the *Entomostraca* of distant lands, but may also be utilized with profit in the laboratory study of our native species, since it offers a ready means of control of the supply of living forms both as to time and numbers. It can be used with equal profit in the study of other fresh water organisms used in laboratories of instruction, such for example as *Amœba*, *Paramœcium*, *Stentor*, *Vorticella*, the rotifers, and fresh water nematodes. A stock once started may be kept from year to year by allowing the aquarium to dry up slowly and keeping the dried bottom deposit for seeding new aquaria.

C. A. K.

Wallengren, Hans. Ueber das Vorkommen und die Verbreitung der sogenannten Intestinaldrüsen bei den Decapoden. Zeitschr. f. wiss. Zool. 70: 321-345, 12 figs., 1901.

Intestinal glands opening into the digestive tract (œsophagus and hind gut) may be demonstrated in *Crustacea*,

such as the crayfish and lobster, as follows: The digestive tract is dissected out, cut open and stretched out upon a wax plate, washed for ten minutes in distilled water, then placed for ten minutes in the dark in a 0.25 per cent. solution of silver nitrate. In about this time it becomes white and opaque, and should be removed to distilled water, in which it is washed for several hours. It is then brought into the sunlight. As soon as the surface becomes slightly browned the tissue is transferred to glycerin for final examination. The openings of the

glands, and sometimes the ducts for a short distance, are blackened, and appear with great distinctness.

C. A. K.

**Kerschbaumer, Fritz.** Malaria, ihr Wesen, ihre Entstehung und ihre Verhütung. Pp. vii, 170. 12 Taf. Wilhelm Braumüller, Wien und Leipzig, 1901. 7 Marks.

The title indicates the scope of this paper, which is especially detailed in its account of the life history of both

*Culex* and *Anopheles*. The following data concerning the habits of *Anopheles claviger* at Rovigno on the Adriatic may prove of service to those who wish to make laboratory studies of the mosquito host of the malarial parasite in America. The eggs are usually of a bluish color (yellowish brown by transmitted light), and when first laid are arranged in rows upon the surface of the water with their long axes parallel, or occasionally in radial or astral groups. They soon shift their position on the slightest disturbance to the sides of the aquarium, where they assume a radial position with reference to its center. The maximum number of eggs is about 150, and they are usually laid in the early morning hours. Unlike *Culex*, the female is easily disturbed by artificial light at such times. The larvæ usually lie in a horizontal position just below the surface film, and dive to the bottom on the least disturbance. Their swift, darting movements are very different from the wriggling motions of *Culex* larvæ. Four moults occur before the pupal stage is reached, and when moulting they lie quietly upon the bottom. They are voracious feeders, attacking even *Culex* larvæ and their own kind. The six stages of growth, egg, four larval, and the pupal, each occupy about an equal part of the period of development, whose rapidity depends in a measure upon the temperature. Below 12°C. development ceases; at 15° to 20°C. it requires four to five weeks; at 20° to 25°, almost three weeks; and at 25° to 33°, eight or nine days. The males do not survive the winter. Fertilized females hibernate in protected crevices about buildings. They emerge and begin to feed early in March. The first generation of larvæ appears in April, emerging early in May. No larva are found in May. The second generation of larvæ appears early in June and continues through the first week of July. From July 7th to July 25th no larvæ were found. Ova for the third generation are laid from July 26th to August 20th, though larvæ continue until September 1st. The fourth generation lasts from the middle of September until the middle of October, reaching a maximum toward the close of the former month. With an average of 150 ova and an equal number of the two sexes, a single hibernated female would leave over 31,000,000 offspring in the fourth brood. The breeding places are quiet, shallow waters, such as pools and puddles.

C. A. K.

**Diederichs, K.** Radula-Präparate. Zeitsch. f. ang. Mik. 7: 29-30, Taf. 1, 1901.

The snails should be killed in boiling water, and when large specimens are used the foot and viscera should be

removed. The remainder of the body is then boiled in caustic potash until thoroughly softened, when the radula and jaws may be removed by washing. The boiling should be repeated in dilute caustic potash until all adherent matter is removed. In small snails the organ is delicate and care must be taken in the boiling and isolation. Unstained radulæ may be mounted in isinglass or glycerin jelly. Mounts in balsam must be stained, preferably in picro-carmin of Ranvier

or Weigert. Large radulæ require 2 to 3 hours of the undiluted stain, the smaller forms 3 to 6 hours of stain diluted with an equal volume of distilled water. Overstains should be reduced with dilute warm caustic potash.

C. A. K.

Spee, F. Graf v. Die Implantation des Meerschweincheneies in der Uteruswand. Zeitschr. f. Morph. u. Anthrop. 3: 130-182, Taf. v-xi, 1901.

Evidence is accumulating that a variety of conditions prevail among mammals in the relation of embryo to uterus.

With ova which attain considerable growth before they become attached to the uterine wall there is no destruction of the uterine epithelium in fixation. In the case of small ova early attached, as in the rat, mouse, guinea pig, and probably also in man, the uterine epithelium is broken down and the egg sinks into the subjacent mucosa. A very important paper by Professor Graf v. Spee, the veteran embryologist, deals with these changes in the guinea pig. The process of implantation of the embryo is accomplished in this animal during the seventh and eighth days after copulation, usually in the second half of the seventh day, and occupies four to eight hours. A full series of ova illustrating all the stages in the process is obtained with difficulty, owing to the uncertainty as to the exact time of fertilization of the egg. A range of about six hours, a period almost equal to that required for implantation, must be allotted for this uncertainty. The embryos are usually found, at this stage, in the antimesometrial angle, or near it, and the whole uterus must be sectioned and examined with a magnification of at least 50 diameters before the one or two ova which it contains can be located.

The author compares the action of the egg upon the uterine walls to that of a parasite upon the tissues of its host. The uterine tissues are relatively passive while the egg is apparently the active agent in the process. The egg has reached a late stage of cleavage and exhibits a small cavity at the time of implantation. The cells of the pole, which later is attached to the epithelium of the uterus, send out processes through the zona pellucida, which soon is cast off from the egg. These ultimately fasten to the epithelium, which is unbroken, and whose subjacent connective tissue and blood vessels are in all respects normal. The naked egg exercises a profound influence upon the maternal tissues. The epithelium gives way, apparently without destruction of cells, though nuclei are crowded and cells are displaced. No cell division is found in the epithelium, and its normal condition is renewed after the egg sinks within it. The adjacent connective tissues exhibit phenomena approaching necrosis; cell division ceases for some distance about the invading egg, the cells degenerate and a fluid gathers about the egg. A well defined sphere of influence can thus be detected in the tissues about the egg. At a later stage an active reaction of the uterus takes place in the development of a granulation zone in the margin of the unaffected tissues, which later has to do with the development of the placenta.

C. A. K.

Webster, J. C. Human Placentation. An Account of the Changes in the Uterine Mucosa and in the attached Fœtal Structures during Pregnancy. 120 pp., 14 figs. and 30 plates. W. T. Keener & Co., Chicago, 1901. \$3.75.

Great differences of opinion have long existed on the subject discussed in this work, which is a presentation, in book form, of Dr. Webster's studies of the

relations of foetal and maternal tissues in the placenta. The work is comprehensive in scope, the discussion is succinct and abundantly illustrated. From comparative and phylogenetic standpoints as well as from his own investigations and those of others, the author concludes that all of the epithelium found on the villi of the human chorion is of foetal origin, thus disposing of the older views of Turner, Balfour, and Ercolani which have found acceptance in most modern textbooks of the subject. A full bibliography accompanies the text.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

**King, Helen Dean.** Observations and Experiments on Regeneration in *Hydra viridis*. Arch. f. Entwicklungsmech. 13: 135-178, 1901.

This paper is a direct continuation of previous work on regeneration in *Hydra*. The present author endeavors to work out in detail important points

which have been left unsettled by other investigators. The experiments were of two sorts; (a) experiments on regeneration after simple injury, and (b) grafting experiments, in which parts of two or more individuals were united.

The technique of the operations is worthy of mention. In all the experiments the common green hydra was used. All the experiments were made in a shallow glass dish, bearing on the inside a thin coat of paraffin. The animals were transferred to this dish and after they had become attached by the foot cuts could be made in any desired direction with a sharp scalpel. "In all the grafting experiments a fine glass thread was used to hold the parts of the graft together, as bristles or hairs when delicate enough for this purpose are very flexible and not readily pushed through the tissues without lacerating them. By heating small glass tubing over the blowpipe flame it is easily drawn out into a thread which can be made very fine and still firm enough to pass readily through the tissues. The glass thread was always left attached to a piece of the larger tubing which served as a handle in grafting, thus making the use of forceps unnecessary. After the pieces of hydra had been brought together, the part of the glass thread containing the graft was broken off from the handle and one end stuck obliquely into the paraffin. The graft was therefore surrounded on all sides by water, and, if the parts had been brought in close contact in the beginning of the experiment, they so rarely separated that at least nine-tenths of all the grafts were successful. In from one to two hours the components of the graft were firmly united. The glass rod was then removed and the graft transferred into a small glass dish containing fresh spring water." In the dishes containing the hydras green water plants were always kept and small crustacea (*Daphnia*, *Cyclops*, etc.) and *Paramecia* were given as food.

Only a few of the more striking and important results of the experiments can be summarized here, while for the numerous interesting details recourse must be had to the original. Regarding the problem of the number of tentacles regenerated after injury the author concludes that both the size of the individual and the size of the hypostome are important factors in determining the number of tentacles in any given case. Double headed hydras were obtained by splitting an individual longitudinally at the oral end. Such double headed individuals always regenerated more tentacles than the original number. The two polyps formed by longitudinal splitting always finally separate and each becomes a com-

plete individual. Double footed hydras can be produced by longitudinal splitting of the aboral end of the normal individual. If two individuals are grafted together with either the oral or the aboral surfaces together and then each component is cut off close to the line of junction, a head will form on one cut surface and a foot on the other, the polarity thus being reversed in one component. When pieces from five hydras are grafted together a single individual is not formed, nor can each component of such a graft maintain its individuality and form a complete polyp. Instead, as many individuals are formed from such a graft as there are heads produced. If the component parts of a graft are of nearly the same size, each part tends to maintain its individuality and produce tissue like itself, while if one component is markedly smaller than the other the smaller part is usually resorbed.

R. P.

**Rothberger, J. C.** Ueber die gegenseitigen Beziehungen zwischen Curare und Physostigmin. Arch. f. d. ges. Physiol. 87: 117-169, Taf. III and IV, 1901.

The author finds as a result of an extended series of experiments on frogs, cats, rabbits and dogs that there is a complete and mutual antagonism

between the drugs curare and physostigmin in respect to their physiological action. A muscle which has been paralyzed through the action of curare can again be set into activity within a few seconds after an injection of physostigmin into the circulation. In case of an isolated muscle preparation the physostigmin may be introduced through the artery supplying the muscle itself, with the same result. An animal completely paralyzed with curare regains entire power of movement after injection of physostigmin. The muscles which are the last to become paralyzed by curare, e. g., the diaphragm, are those which first regain irritability as a result of the action of the physostigmin. The effectiveness of the physostigmin is independent of the duration of the curare paralysis. A very curious result is observed if a mixture of the two drugs is injected into the circulation. They do not neutralize each other, but instead complete curare paralysis occurs, and in about a half hour spontaneously disappears, the animal regaining its normal activity. Both drugs act on the endings of the motor nerves in the striated muscles.

The author points out a practical application of these interesting results which is likely to prove of technical value. This is the use of curare in place of an anæsthetic in difficult operations in physiological experiments. Immobilisation can be made very complete and the animal subsequently revived by the injection of physostigmin. The procedure recommended is as follows: the animal is quieted by injection of about half the amount of curare necessary to produce complete paralysis, and an artificial respiration apparatus is inserted through the larynx into the trachea, and the operation is begun. When the operation is practically completed 2 to 3 mg. of physostigmin and at the same time 2 mg. of atropin are injected. In about ten minutes the respiration apparatus may be removed, the wound in the meantime having been closed with stitches. This method has been found to give excellent results in practice.

R. P.

**Hedin, S. G., und Rowland, S.** Ueber ein proteolytisches Enzym in der Milz. Zeitschr. f. physiol. Chem. 32: 341-349, 1901. Untersuchungen über das Vorkommen von proteolytischen Enzymen im Thierkörper. Ibid. 32: 531-540, 1901.

Enzymes which dissolve albumens have been extracted from the fluid obtained by crushing and pressing finely divided portions of various organs and tissues of the animal body. Such proteolytic

enzymes have thus far been obtained from the spleen, lymph glands, the kidney, the liver, skeletal muscle, heart muscle, and blood corpuscles. The enzymes from the spleen, lymph glands, kidney, and liver are most active in acid solution, while that obtained from skeletal muscle tissue is relatively weaker and shows no marked difference in its activity when in acid, neutral, or alkaline solution.

R. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

The American Society of Bacteriologists held its third annual session at Chicago on December 31, 1901, and January 1, 1902. The meetings were very largely attended. A large number of valuable papers were read at the meetings, and abstracts of a few of them, especially those relating to new suggestions as to bacteriological methods, are given below:

**Novy, F. G., and Freer, P. C.** On the Germicidal Action of the Organic Peroxides. University of Michigan.

The work reported in this paper was done in attempting to discover the reason why the poisons of insoluble metals

have a checking action upon bacteria growth, and resulted in the discovery of a new germicide of rather extraordinary properties. In the course of the experiments the germicidal action of the various peroxides was studied. The results showed that some of these bodies, such as acetone peroxide and dibenzoyl peroxide, are wholly inert.

On the other hand, solutions of diacetyl, benzoyl-acetyl, and of benzoyl-hydrogen peroxides, and of phthalmonoperacid, exert pronounced and even remarkable germicidal properties. With reference to diacetyl peroxides and benzoyl-acetyl peroxide, it was shown that the bodies themselves are chemically and bacterially inert; but on contact with water they undergo hydrolysis and give rise to the extremely energetic acetyl-hydrogen and benzoyl-hydrogen peroxides.

A solution of these peroxides (1:3000) is capable of destroying all pathogenic bacteria, and even such resisting spores as those of the potato bacillus, within one minute. Cholera and typhoid germs added to tap water are promptly destroyed by the addition of one part of peroxide to 100,000 parts of water. The authors point out the probable value of these peroxides in the prevention and cure of these and allied diseases. The destruction of bacteria in the mouth and saliva takes place with extraordinary rapidity and the reagents have shown themselves useful in diseases of the mouth.

A full paper upon this subject will appear in the *Journal of Experimental Medicine*.  
H. W. C.

**Hiss, P. H.** Contribution to the Physiological Differentiation of Pneumococcus and Streptococcus, and the Methods of Staining Capsules. College of Physicians and Surgeons, New York.

These authors pointed out the difficulty of distinguishing between these organisms in all cases, and have suggested a new method of separating

them. The method consists in the use of new culture media as follows:

Two culture media are used with the following composition: 1. Distilled water, 2 parts; ox serum, 1 part; normal sodium hydroxide, 0.1 per cent.

2. Distilled water, 2 parts; ox serum, 1 part; and inulin, 1 per cent. In each of these media acid is formed by pneumococci at 37° C., and a solid, yellowish white coagulum results. The streptococci, however, do not form acid in these media, and no coagulation results.

The author also devises a new method of demonstrating capsules upon these two coccus forms. The method described is as follows:

The organisms are grown on ascitic serum agar with 1 per cent. glucose. The bacteria are spread on a cover-glass by mixing with a drop of serum. They are then dried in the air, fixed by heat, stained for a few seconds in a one-half saturated aqueous solution of gentian violet, washed with one-fourth per cent. solution of potassium carbonate, then mounted and studied in this solution. This gives a good stain for capsules of pneumococcus in the blood of animals.

A second method of staining makes it possible to mount the preparation in balsam. It is as follows: A 5 per cent. or 10 per cent. solution of gentian violet or fuchsin is used. This is placed on the dry and fixed cover-glass preparation and gently heated until steam rises. The dye is washed off with a 20 per cent. solution of copper sulphate ( $\text{CuSO}_4$  crystals) and the preparation is dried and mounted in balsam. By these methods most streptococci have been shown to have capsules.

H. W. C.

Hill, Hibbert W., M. D. "Hanging Block" Preparation for Microscopic Observation of Developing Bacteria. Boston Board of Health, Bact. Lab.

The writer cuts a cube of nutrient agar from a Petri-dish full of solidified jelly. The organism to be examined, as an emulsion in water from a solid culture,

or as a drop of broth from a liquid culture, is spread upon the upper surface of the agar, as in making an ordinary smear preparation on glass. After drying the cube at  $37^\circ \text{C}$ . for 10 minutes, a clean coverslip is applied to the inoculated surface and sealed in place by running a little melted agar round the edges of this surface. The coverslip is then placed over the opening in the moist chamber, the agar block lowermost, and the microscope focused upon the bacteria. For organisms growing best at  $37^\circ \text{C}$ . some form of warm chamber is necessary. The writer describes two such warm stages, devised by himself, and a very simple method of securing a circulation of warm water through them.

H. W. C.

Gorsline, C. S. An Improved Method of Making Collodion Sacs. University of Michigan.

The use of collodion sacs in bacteriological investigations has come to be quite common in recent years. The methods

of producing these sacs have been somewhat difficult. Gorsline has added one point of simplification to the methods that have previously been used, which renders the production of such sacs a very much simpler operation than it has been hitherto. Indeed, it is now, by the new method, an extremely simple matter to produce collodion sacs of practically any desired size. The method which Gorsline has devised and described is as follows:

A long tube is prepared, closed at one end, except for a minute perforation at the bottom, from 2 to 4 millimeters in diameter. A wide mouthed bottle, holding 6 to 8 ounces, is filled about one-third full of colorless collodion of the common strength. The first step is to touch the end of the tube to the surface of the collodion, thereby obtaining a film of collodion over the small opening, but allowing none to enter the inside of the tube. This is allowed to dry for a few moments and harden. The bottle containing the collodion is then inclined as much as possible without spilling, the tube inserted into the collodion and slowly rotated, allowing the lower fourth of the tube to be immersed in the collodion.

The tube is withdrawn from time to time to allow a partial drying to take place; the operation being repeated until the desired thickness is reached. Two or three coats thus obtained are commonly sufficient. As soon as the collodion has set, the tube may be immersed in water at about  $25^\circ$ , to hasten the drying of the sac. When this is ended, distilled water is put into the open end of the tube, and by applying the mouth, the water is forced through the perforation in the tube at the bottom, carrying the collodion ahead of it. By blowing into the tube the water is caused to creep in between the sac and the tube, gradually separating the collodion sac from the glass, the process being aided and made to progress evenly on all sides by twisting and shifting back and forth the free end of the sac. When the water has risen to the top of the sac, the latter slips off from the glass easily into the hand. The water by its pressure not only releases the sacs, but tests it for weak places and for perforations.

Collodion tubes may be thus made from one-sixteenth of an inch to two inches in diameter, and prove to be extremely useful, not only for bacteriological experiments, but for dialyses. The rapidity with which the process of dialysis goes on through these sacs is very great.

H. W. C.

## NEWS AND NOTES.

AMERICAN MICROSCOPICAL SOCIETY.—Descriptions of the special apparatus used in collecting the samples and of the methods employed are given. The physical examination includes temperature, appearance, turbidity, color, and odor. The factors of the chemical analysis are nitrogen as albuminoid ammonia, nitrogen as free ammonia, nitrogen as nitrites, nitrogen as nitrates, residue on evaporation, loss on ignition, chlorine, hardness, alkalinity, incrusting constituents, iron, oxygen consumed, dissolved oxygen, and carbonic acid. The microscopical examination determines the number and kinds of microscopic organisms present, together with the amount of amorphous matter. The number of bacteria present is determined by a quantitative test, the only bacterial organism which is especially looked for being *Bacillus coli communis*. The paper closes with some observations on the microscopic forms which have been found to be of sanitary importance, especially *Asterionella*, *Synedra pulchella*, *Cyclotella*, *Melosira*, and *Anabana*, all of which have been found to be instrumental in the production of unpleasant tastes or odors, or both. The paper is illustrated by four plates, showing views and the plan of the laboratory, and the seasonal variation in number of some of the more important organisms.

The paper on "Methods of Producing Enlargements and Lantern Slides of Microscopic Objects for Class Demonstration," by John Aspinwall, is so full of technical directions that no abstract will do it justice. The writer uses the Paget plate, made in England, and seeks to avoid the glaring black-and-white effect so characteristic of ordinary slides. The object of the method is "to produce photomicrographs of considerable magnification, and yet of great depth of focus, while using lenses of high resolving power."

"On the Distribution of Growths in Surface Water-Supplies and on the Method of Collecting Samples for Examination," by Frederick S. Hollis, is a study of the seasonal distribution of the microscopic organisms found in the eight reservoirs of the Metropolitan Water Works. These reservoirs vary from fifteen to sixty feet in depth and contain from a third of a billion to seven and one-half billion gallons of water. The organisms were counted in samples taken from the surface, mid-depth, and bottom. It was found that in general the number of organisms present was greater in the reservoirs through there was a fairly marked flow of water as compared with those in which the currents were not so noticeable. At the time of the autumn and spring overturn the organisms are found to be very evenly distributed vertically throughout the water. The paper is accompanied by four charts showing the variation in abundance, during five years, of the organisms at the surface, mid-depth, and bottom, for several of the reservoirs, the variation of temperatures in Lake Cochituate for 1896, and the variation in the color of the water of the same lake during that year.

University of Rochester.

CHARLES WRIGHT DODGE.

(Continued.)

Mr. Harbert Hamilton has started on a scientific exploring and collecting trip in the vicinity of Santiago, Cuba. Collections will be made in all branches of natural history, the bulk of which will go to the New York Botanical Gardens, the American Museum of Natural History, and the Academy of Natural Sciences of Philadelphia. Specialists or institutions desiring material in any branches direct from the locality are invited to correspond with Mr. Hamilton at Santiago, Cuba.

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It is proposed to hold a summer school of chemistry and biology at Wesleyan University, Middletown, Conn., between July 1 and 31, 1902. Lectures and laboratory courses will be offered, embracing methods employed in the investigation of food and nutrition, food analysis, dietary studies, qualitative and quantitative analysis, chemical phenomena at low temperature, bacteriology, etc. Information may be obtained from Professors F. W. Nicolson, W. O. Atwater, W. P. Bradley, or H. W. Conn.

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Prof. W. W. Rowlee, of the botanical department of Cornell University, and Prof. J. C. Gifford, of the College of Forestry, have gone on an expedition to Cuba to study the botany and forests of Western Cuba and the Isle of Pines.

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The president of the Association Internationale des Botanistes has appointed the following American editors for the *Botanisches Centralblatt*:

D. H. Campbell, Stanford University, California.—*Morphology*.

C. J. Chamberlain, University of Chicago.—*Cytology*.

D. T. MacDougal, New York Botanical Garden.—*Physiology*.

G. T. Moore, Department of Agriculture, Washington, D. C.—*Algæ, Lichens, Archegoniates* (systematic).

D. P. Penhallow, McGill University, Montreal.—*Paleobotany*.

H. von Schrenk, Washington University, St. Louis, Mo.—*Fungi* (systematic) and *Vegetable Pathology*.

William Trelease, Missouri Botanical Garden, St. Louis, Mo.—*Phanerogams* (systematic).

For the coördination of the editorial work, the two editors last named have been asked to serve respectively as secretary and chairman of the American board.

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We have received a copy of the constitution of the Montana Academy of Sciences, Arts and Letters, recently organized at the University of Montana. The annual meetings of the academy will be held between Christmas and New Year, with additional summer meetings as desirable. Information regarding the Academy may be obtained from Morton J. Elrod, president.

## QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

17. What is the method and formula for the sulpho-carbolate of zinc in glycerol as a mountant, and for what is it particularly useful?—V. A. L.

18. Will any reader who has had *practical* experience give really reliable formulæ for decalcification fluids for teeth, so that the pulp is left in good condition for study and staining?—V. A. L.

19. On p. 1422, in Dr. Harris' formula for elastic stain, mercuric *acid* is given. What is the synonym or formula for same? Merck gives no such.—V. A. L.

20. In formula for Harris' hæmatoxylin solutions no agent is given to prevent mould, which I find is very rapid and in quantity.—V. A. L.

21. Will anyone state how the gas can be prevented from going out with thermostat and burner under incubator? Just as soon as it steadies at 37°C. it goes out, invariably within a short time. If a Koch automatic burner is used, as soon as it is steady the expanding coil cools so the arm drops and shuts off the gas. The tube is attached to the main pipe from the meter.—B. E. B.

22. What is a simple method for the examination of blood for clinical purposes, with special reference to counting the white cells, differential count, estimating relative proportions, and obtaining color coefficient. Also what is Hayem's method of expressing the amount of hemaglobin in normal red corpuscles (per cent.)?—J. H. W.

23. Referring to Dr. Goldhorn's new blood stain, reviewed on page 1635 of the JOURNAL, the following questions have been asked:

(a) Should the solution be made up to any definite quantity after evaporation, or is it the intention to evaporate it down to concentration?

(b) Is it better to decant from the thick sediment before attempting to neutralize the reaction?

(c) Is not the sediment composed largely of lithium carbonate, which would prolong the operation of acidification if left in?

(d) After bringing the solution to an acid reaction, is it better to neutralize with the powdered lithium carbonate or the solution; in other words, is there any point in keeping the solution nearly at saturation, or will it act just as well if somewhat diluted during the process of correcting the reaction?

(e) Is there any objection to filtering after the reaction is corrected?—E. H. B.

### REPLY TO QUESTION No. 23.

(a) As to quantity: Make 300 c.c. if you use an ordinary small water bath heated by a single Bunsen burner.

(b) As to decanting: Yes, decant; or, better still, run through cotton.

(c) As to the residue's nature: It is largely lithium carbonate; get rid of it as suggested, sub. 2.

(d) As to use of lithium: Use it in saturated solution; you may dilute as suggested in my description.

(e) As to filtering: Do not filter through filter paper; use some cotton in a funnel.

L. B. GOLDHORN.

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## **The Biology Laboratory of Vassar College.**

The Biology Laboratory of Vassar College occupies a part of the "New England Building," a gift to the college from alumnae living in New England, and which was completed in December, 1901. The building faces north. Its main part is rectangular in form, measuring 86 x 57 feet, and comprises two

FIG. 1.—Front View. New England Building.

stories and a basement. To this there is added in the rear a semi-circular extension, with a depth of 32, and a width of 44 feet, and running to the same height as the main part of the building. The materials of construction are Bedford stone, brick, and terra cotta. In planning the building, especial care was

taken to provide abundance of light, and the windows are numerous and large. (Fig. 1.) The architects were Messrs. York and Sawyer of New York.

Heating is by means of direct and indirect radiation, and illumination by gas. Gas is supplied to each student's desk, for use either with a Welsbach or for a micro burner for histological or other work requiring a flame. At present the only electricity available is for projection purposes in the large lecture room.

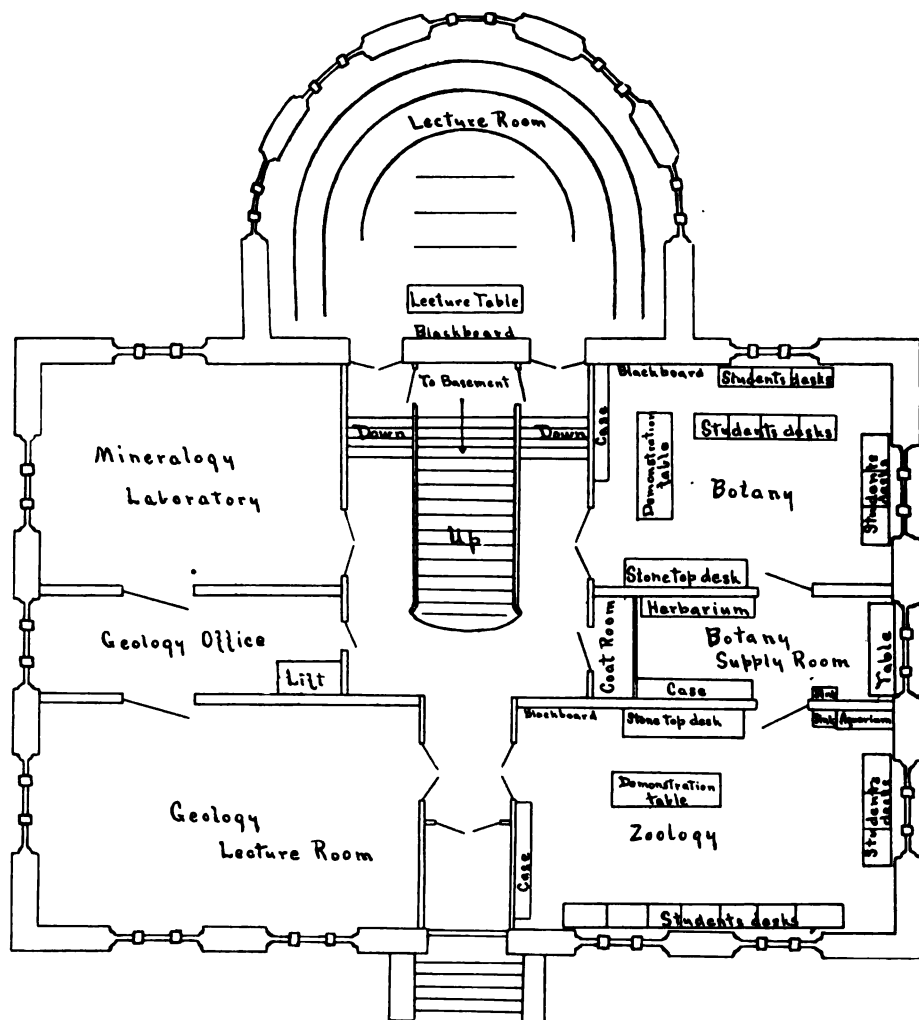


FIG. 2.—First Floor.

The building is wired, however, and as soon as proper arrangements can be made, electricity for illumination will be supplied to each desk. For the present, artificial light is supplied by Welsbach lamps, which can be attached to the gas cocks on the desks. Even the best lighting of this sort is, for microscopical work, so very unsatisfactory, that we shall make as little use of it as possible.

Thus far we have been able to arrange our work so that all microscopical work shall be done by daylight.

The main entrance opens into the large central hallway (see diagram of floor plan), from which a stairway leads to the second floor. On either side of this stairway is a passage, and a short flight of steps leading down to the level of the platform in the large lecture room, which is used by all the departments having work in the building. This room seats 120, and is furnished with opera

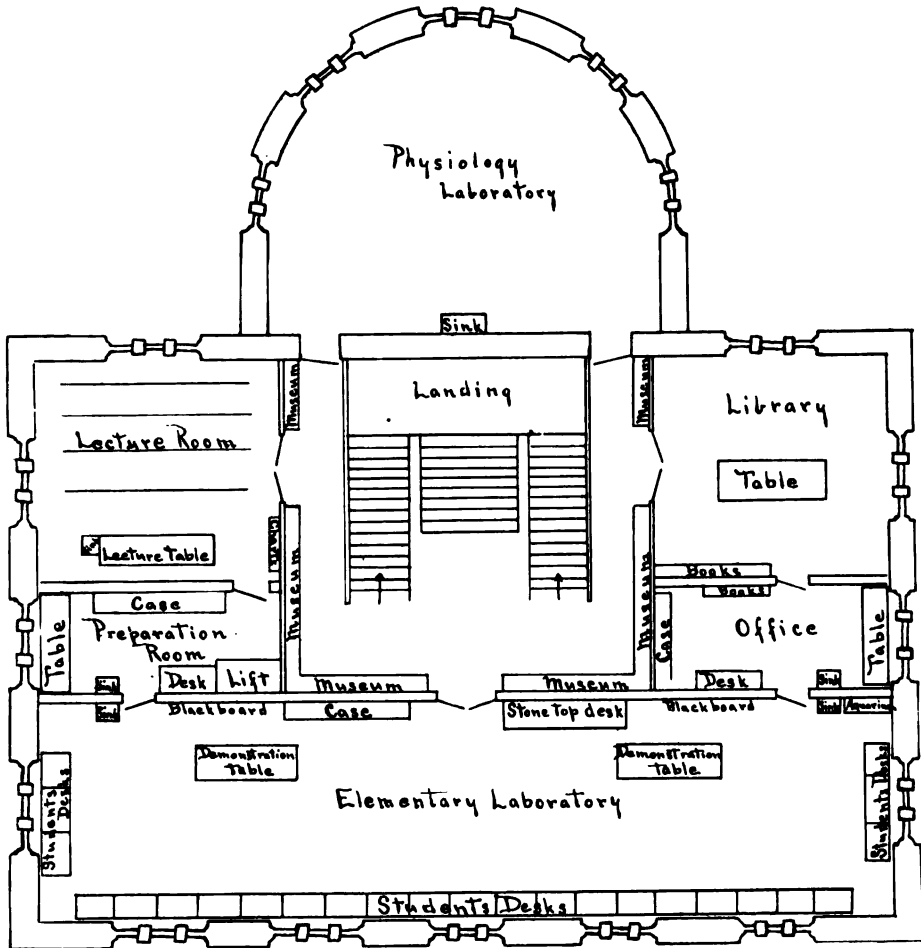


FIG. 8.—Second Floor.

chairs with folding writing arm. Two frames hung by weights so as to slide up and down in front of the blackboard, behind the lecture table, are for hanging charts. This room is to be equipped with an electric lantern for projection purposes.

From the rear of the main hallway, stairs lead to the basement.

For all laboratories we have adopted a uniform pattern of furniture, which can best be described here. The plan of the students' desks, as seen in front

view, is shown in the outline drawing, Fig. 5. These are 2' 6" high, 3' 8" long, and 2' 6" wide. The working space for the student is, therefore, 30 x 42 inches. The dimensions of the drawers and closets as given in the drawing are inside measurements. On the right, at the bottom, is a closet intended to hold the student's microscope, wash bottle, etc. Above are two drawers, the upper, the deeper, measuring 6" in depth (height). At the bottom of this drawer is an inch board, with holes of just the right size to hold reagent bottles. For courses

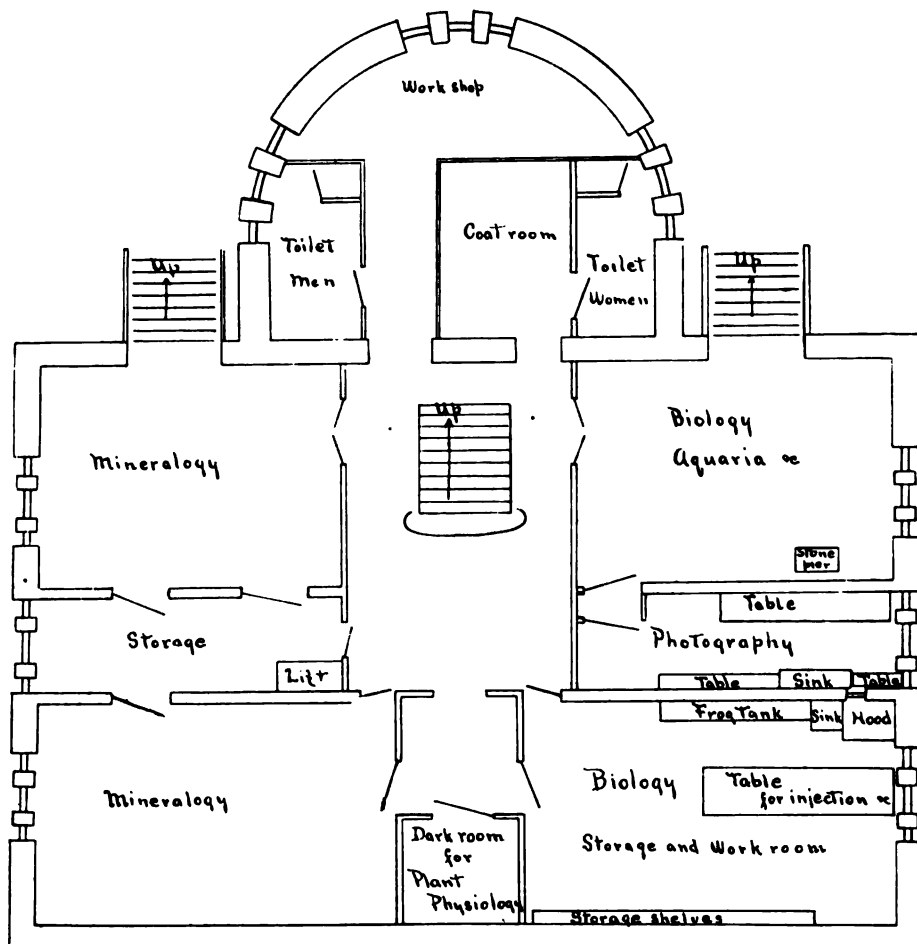


FIG. 4.—Basement.

where comparatively few reagents are used, it is proposed that all bottles shall be kept in these drawers. For histological courses, where a good many reagent and staining jars are used, it probably will not be practicable to keep all bottles in the drawer, but whenever possible it is proposed to do so. Not only are the bottles themselves better protected, and freer from dust, but the general appearance of the tables is very greatly improved. The plan has been tried for a number of years in another laboratory, and has given excellent satisfaction. A

shallow drawer underneath the center of the table, and another between the reagent drawer and the closet, afford room for note books, drawing materials, etc. In order to obviate the constant annoyance resulting from loss of keys, each closet is provided with a combination lock of the kind used in gymnasium lockers. The desks are of ash, the tops blackened, and the sides treated with a preparation of wax. Ash was used in all the furniture to match the interior finishing of the rooms. It is our experience, however, that some other wood should be chosen for tops. Under the paraffining process the grain of the ash is such that it is liable to "rub up" and roughen the surface.

The above description is that of the unit desk. To economize cost as well

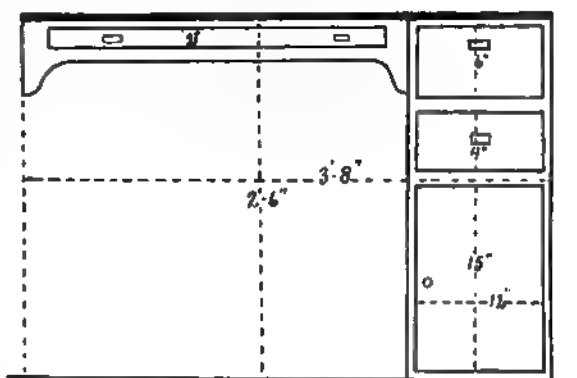


FIG. 5.—Plan of front of student's desk.

FIG. 6.—Preparation and Assistant's Room.

as space, there is no separate support at the left in all cases, but that end is supported against the end of the next desk, as shown in the photographs. A half round strip covers the line of junction, and runs along the back of the desks. In this way any desired number of units can be combined. The last desk in the row is supported by a special end.

Each desk is provided with an adjustable stool. In my experience no other laboratory seat is satisfactory.

Desks for holding paraffin baths, sterilizers, etc., are 3' 4" high and 2' 6" wide. They have closets at either end with three rows of drawers down the middle, and are arranged to stand against the wall. For desks of this sort fire-proof tops are essential, and we have adopted soapstone in preference to slate, on account of the tendency of the latter material to crack under the heat of a sterilizer. In the laboratories, these desks are 10' 6" long. A smaller one in the preparation room is 5' long, but with the other dimensions the same as these.

The soapstone tops of these desks, as well as those of the sinks, are filled with oil which turns them black, thus matching well the tops of the tables, and shows stains much less than would the natural color of the stone.

FIG. 7.—Botany Laboratory.

Demonstration tables standing in the laboratories, behind the students' desks, are 31" high, 34" wide, and 8' long. Each has a closet at either end with two rows of drawers in the middle. The tops are blackened like those of the students' desks.

Wall cases are 8' 6" long, and 8' high. Below are closets, 3' high and 2' deep. Above are cases with glass sliding doors 5' high and 18" deep. The latter have adjustable shelves.

For convenience in keeping a card catalogue of apparatus and other equipment, each piece of furniture is lettered, and each shelf and drawer in it is numbered. For this numbering we have adopted a small brass thumb tack, with black enameled number, such as is used by manufacturers of window screens.

These are easily attached, and are the most satisfactory arrangement for this purpose that I have seen.

Lecture tables are 10' long, with a closet and drawer at either end, and a solid back. The tops are blackened like those of the other furniture.

All sinks are of soapstone. Water is supplied to the aquarium sinks in the usual way—that of a pipe running longitudinally, 3' above the sink, with a series of small “pet-cocks” on the under side. A number of these small cocks arranged over the sink in the preparation room, provide small streams of water for washing out purposes. On each floor a small water heater fastened over the sink furnishes hot water.

The general arrangement of the rooms is given in the accompanying diagrams of the ground plan of each floor. At the right of the main entrance on

FIG. 8.—Corner of Elementary Laboratory showing student's desk and sink.

the first floor is the laboratory for advanced Zoölogy and Embryology. This has ten students' desks facing the windows, with arrangements for a second row behind the first. The arrangement of the other furniture is shown in the diagram. Not shown there, is a small corner set of shelves, used for holding reagent bottles.

Behind this is the Botany supply room, containing the herbarium, a case for supplies, and a desk for microscopical work. This latter is of the same height and width as the students' desks, but extends in front of the window for the whole width of the room, being thus 10' long. A row of drawers runs down the middle, leaving working space on either side. A similar table is in the private office and in the preparation room on the second floor. (See photograph, Fig. 6.)

Beyond the supply room is the Botany laboratory, fitted with the same kind of furniture as the other.

On the opposite side of the hallway from these rooms, are those occupied by the Department of Geology and Mineralogy.

On the second floor the large central hallway contains a row of museum cases, intended to hold the collections, models, etc., used in the work of the department (see photograph, Fig. 9). The main part of the museum collection is in the College museum, where they are available for reference.

The whole of the north end of the second floor is a laboratory for Elementary Biology (Fig. 10). This room measures 80 x 19 feet, and, when fully equipped, will accommodate thirty-nine of the students' desks above described. Behind

FIG. 9.—Upper Hall with Museum Cases.

this room on the west is the office and private laboratory, and behind this the library room.

Behind the large laboratory on the east is the preparation room, provided with soapstone top desk, sink, wall case and table (see photograph, Fig. 6). Behind this is a small lecture room, seating thirty-two. The lecture table in this room is supplied with gas and water. A large chart frame slides in front of the blackboard.

The Department of Physiology occupies, as a laboratory, the room on this floor directly over the large lecture room.

The basement has a cement floor, but the walls and woodwork are finished precisely as on the other floors, so that the rooms are available for many purposes. The front room has a frog tank, sink with water heater, still for water, and a hood, with ventilating flue. A large table in front of the window is supplied with gas, and is to be used for injections and similar operations.

The photographic room gets ventilation through the same flue that ventilates the hood, and is lighted by an outside window which can be shut off, and by a lamp in the hood, the light coming through a window in the partition.

Water connections have been made in the room behind this photographic room, so that it can be, if desired, fitted later with aquaria.

FIG. 10.—Elementary Laboratory.

Coat and toilet rooms are on this floor.

A small dark room at the front of the basement is to be used for experiments in plant physiology. It is to be provided with apparatus for maintaining constant temperatures. A good deal of this work is, however, carried on in the conservatory, where tables have been arranged for that purpose.

Vassar College.

AARON L. TREADWELL.

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A TEST TO DISTINGUISH METHYLEN BLUE FROM METHYL BLUE.—If ammonia water is added to a dilute solution of methyl blue, the solution is decolorized; whereas, a very highly diluted solution of methylen blue is not decolorized by the ammonia water. There are also two distinct kinds of methylen blue which should not be confused; the methylen blue used for medicinal purposes and the methylen blue used for staining purposes.—*Merck's Archives*.

## A Convenient and Economical Cabinet for Microscopical Slides.

There are many cabinets on the market which are admirable, and those who are not limited by means and space can, no doubt, find among these something suitable. On the other hand, some persons, I have no doubt, still keep a large number of slides in the common wooden boxes which are so generally used for that purpose, and it is presumed that they have experienced the inconvenience of not being able to find the right box and the desired slide just at the moment when it was most wanted.

The accompanying photograph represents a case which I have had constructed for my laboratory to hold the slide boxes just mentioned. As will be seen, the case holds four drawers, but it might be made of any size. The drawers are all  $25\frac{3}{4}$  inches deep and 20 inches wide outside and are made of  $\frac{5}{8}$ -inch lumber. The top drawer is  $3\frac{1}{2}$  inches deep inside, and in the front portion there are three spaces each 5 inches wide for catalogue cards. The remainder of this drawer can be used for extra cards and labels. The other three drawers are of uniform size and are 7 inches deep inside. At the back of each there is a space  $5\frac{1}{4}$  inches wide and as long as the width of the drawer. This is left so that the drawer will still hang firmly in the case when withdrawn far enough to allow of the removal of the last row of slide boxes. The remainder of each of the three lower drawers is divided into 50 spaces, each  $1\frac{1}{8} \times 3\frac{1}{2}$  inches, by

### Cabinet for Microscopical Slides.

partitions made of  $\frac{1}{4}$ -inch lumber. The spaces between the partitions are just large enough to receive comfortably one end of the ordinary wooden slide box. Since each box holds 25 slides and each drawer 50 boxes, the case will accommodate 3750 slides. The drawers are lettered A, B, and C and each box is lettered and numbered, beginning with the inner left hand box of each drawer. The slides are also lettered and numbered to correspond with those of the drawer and box and, if desired, may be numbered from 1 to 25 in each box to correspond with the numbers in the backs of the boxes. A card catalogue is

made of the slides so that it is but a moment's work to find the one wanted. When a number of slides have been used it is an easy task to return them to their proper places, a thing which will be appreciated by every teacher. By such a method an inexperienced laboratory assistant or student can readily find any desired slide and return it to its proper place.

The slide boxes may be used just as they are purchased, but it is desirable that the lids should be hinged, for they will become exchanged and not fit well. Brass hinges such as are frequently used on cigar boxes can easily be attached, also a brass catch to keep the lid closed. I have hinged a number of lids by simply glueing a strip of muslin to one edge of the lid and box.

For all practical purposes this slide case answers well. It is compact; the slides are kept face up; each slide can be numbered so that it may be quickly found and is just as easily replaced; the boxes are convenient for carrying the slides from the case to the microscope; and it is economical. The case here described is made of oak, neatly paneled, and without the slide boxes cost \$15.00.

Minn. State Normal School, Mankato.

ULYSSES O. COX.

### Carbol Fuchsin in General Botanical Work.

The desirability of students at least staining their own sections needs no comment. The slow action of many of the most valuable stains makes this almost impossible in cases where the time available for laboratory work is so limited that everything must be done under more or less pressure. Having had occasion to search for a stain that would bring out the cell structure in the stem of *Capsella*, I happened to try carbol fuchsin made by Ziehl's formula:

Water	-	-	-	-	-	-	100 c. c.
Alcohol	-	-	-	-	-	-	10 c. c.
Carbolic acid	-	-	-	-	-	-	5 gr.
Fuchsin	-	-	-	-	-	-	1 gr.

and was surprised to find that after one or two minutes in the stain and subsequent rapid transfer through the alcohols and xylol into balsam, the specimen showed a differentiation almost as decided as that produced by hæmatoxylin and safranin. Lignified cells showed a red nearly as brilliant as those stained several hours in safranin, while cellulose walls are very clearly brought out in light purple.

I have since tried this stain with a variety of other stems, both mono- and dicotyl, and the results have been in every case satisfactory. In addition to its quick and good differentiating power, it is very transparent, even moderately thick free hand sections being quite satisfactory in this respect.

This stain may be in use in other laboratories for this purpose, but I do not know of one in which it is so used, nor have I ever seen it referred to as a stain of general value, except in bacteriological work. I regard it as a valuable addition to the stock of staining fluids suitable for a beginner in plant histology.

University of Georgia.

JOHN P. CAMPBELL.

**LABORATORY PHOTOGRAPHY.**

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

**PHOTOMICROGRAPHY.****IV. Focusing the Instrument.**

With low powers and a good light, focusing by means of the image on the ground glass is not difficult so long as the operator can reach the focusing screw.

Fig. 1.

Fig. 2.

If the objective used is a 35 mm. or one of lower power and a camera extension of four or five feet is to be used, for the purpose of getting depth of focus with increased magnification, it becomes necessary to control the coarse adjustment screw by some means. We used a rod on the camera table with pulley attachment. This is of course detached when high powers are used. A lens is often used to ascertain the focus on the ground glass; it should for this purpose have telescopic mounting or rest on legs so that it will be at right angles with the image and at the same time remain firmly in focus; but the more experience one has the more I think he will rely on naked eye appearance. The beginner should study the focus carefully with and without a ground glass in the path of the rays, with and without a hand lens and with diaphragms of all sizes; he should compare the appearance on the ground glass with the appearance under the microscope with correct illumination; in no other way can he ever learn to correctly illuminate and focus his object for photography.

It is only in photomicrographs of a thousand diameters and upwards that the real difficulties of focusing begin. It is a good plan when an immersion exposure has been made to immediately replace the ground glass to see whether or not the focus has by any accident changed during the exposure. Real progress can also be made by faithfully comparing every negative that shows defective focus with the image on the ground glass.

I would especially recommend as a valuable study in sharpness of the image an object which has clear detail in several different planes, as for instance a Golgi nerve cell or a kariokinetic figure with iron-haematoxylin stain; focus on some clear detail in a middle plane; having made an exposure compare carefully the negative with the image on the ground glass; the relationship between

Fig. 3.

Fig. 4.

the negative focus and the ground glass appearance can in this way be correctly mastered. The beginner should stick to this practice until he can pick out his detail in such a slide and correctly reproduce it in his negative. Figs. 1 and 2 show such a study; the centrosome in one cell of Fig. 1 is sharp and in the other cell of Fig. 2.

Every one who attempts to make photomicrographs meets the difficulty of focusing; with a bright light, a low power, and a means of controlling the adjustment screws, this difficulty vanishes; the Misses Foote and Strobell of New York City, who have a seaside laboratory at Woods Hole, have met this difficulty in powers up to a thousand in an entirely novel way. Their method is simple, and the necessary apparatus in addition to a good microscope need not cost more than five dollars. The microscope is put in an upright position with

an upright camera of any description. A base board on which the microscope rests, to which are attached two upright posts carrying a camera capable of being extended thirty inches above the object, is all that is necessary. The light used is daylight from a clear sky exactly as one uses it in microscopy, no color screens or cooling cells or field diaphragms; the only substage arrangements are an adjustable Abbe and iris diaphragm. The lenses and eyepieces must of course be the best obtainable for this purpose. By this arrangement the light is not sufficient to enable one to focus on the ground glass. These ladies concluded that there must be some relationship between the focus for ordinary microscopy and that for photomicroscopy at any given extension; by experiment they found that any one can select a spectacle-glass with which if he focuses his instrument, the focus will also be right on the sensitive plate at a particular extension. These glasses can be bought of any optician; they are labeled  $D\frac{1}{8}$ ,  $D\frac{1}{4}$ ,  $D\frac{1}{2}$ , etc., up, and  $D-\frac{1}{8}$ ,  $D-\frac{1}{4}$ , etc., down. With a Zeiss 2 mm. apochromatic objective, a 160 mm. tube-length, a No. 4 projection eyepiece, a camera extension of  $29\frac{3}{4}$  inches, and an operator slightly on the sunny side of thirty, a  $D\ 2$  lens will approximately correct the focus; younger operator, higher number; older, lower. A good photographer can focus his second exposure correctly as follows: get an approximate focus on a middle detail in a thick section having sharp details in several planes; make an exposure and develop the negative; with the negative or a print in hand select an eyeglass that gives the same image as the one seen in the negative; this will be just the glass to use for focusing under the given conditions. In the same way a glass can be chosen for any other assemblage of lenses and conditions. This is the only sort of cheap outfit I have yet seen that I did not regard as too expensive. These ladies have rendered a valuable service to all who desire to practice photomicrography with an inexpensive outfit.

Figs. 1, 2, 3 and 4 all represent stages in the first kariokinetic division of the fertilized egg of *Ascaris*. All are made with a 2 mm. objective and No. 4 projection eyepiece, with a camera extension of 37 inches. All show a sharp focus in the respect in which it was intended to be sharp.

Earlham College.

D. W. DENNIS.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### IV—TETANUS.

The bacillus of tetanus is found in the pus at the point of inoculation. Spread a thin film of the pus on a clean cover-glass held in a Cornet forceps, using a platinum loop, which should be sterilized before and after using. Pass the cover-glass, film side up, three times through the flame of an alcohol lamp or Bunsen burner. Stain with carbol fuchsin, methylen-blue or methyl-violet, putting on the film side of the cover a liberal quantity of the stain selected. Stain from one to five minutes, wash in water, and dry between filter paper and

in the air. Mount film side down in a drop of balsam on a clean slip. The bacillus of tetanus will appear like pins, the spore at one end of the bacillus resembling a pin head.

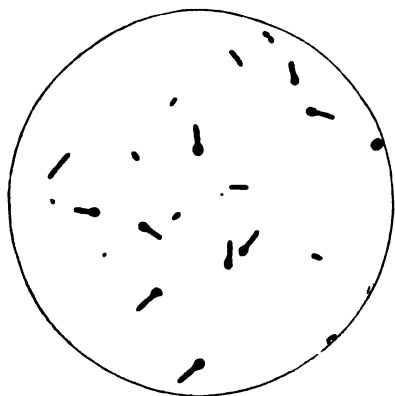


FIG. IX.—Bacillus of tetanus. Culture stained with methyl-violet. Magnified 1200 diameters;  $\frac{1}{8}$ -inch oil immersion objective, Bausch & Lomb compensating photo ocular No. 2.

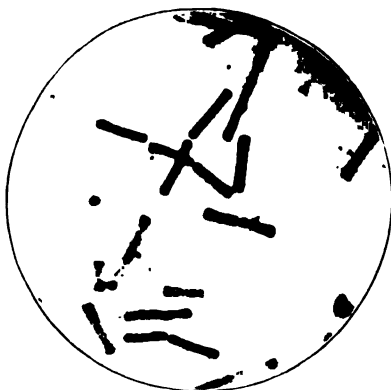


FIG. X.—Bacillus anthrax, from blood. Stained with Loeffler's alkaline methylen-blue. Magnified 800 diameters;  $\frac{1}{8}$ -inch objective, Zeiss projection ocular No. 4.

#### BACILLUS ANTHRACIS—Splenic Fever.

The blood of animals suffering from splenic fever always contains the bacillus of anthrax. To prepare a slide for examination, a drop of blood should be drawn from a thoroughly cleansed surface, preferably the ear, which should be washed with soap and water, then with alcohol, and finally with ether. A blood lancet should be used, as it insures a free flow of the blood. Cleanse two glass slips with alcohol first, then with ether. Apply one to the blood drop so that a half-size drop may adhere to it within about one-half inch of one end. Apply the end of the other slip to the blood so that the edge rests lightly in the blood and in contact with the glass beneath. Draw this slip, which acts as a spreader, gently along the first or blood slip, making a thin spread two-thirds its length. After the film of blood has dried in the air the slip with the film side up should be heated over an alcohol flame or Bunsen burner until it is too hot to hold in the fingers. Cool it partially and reheat. Stain the film with Loeffler's alkaline-blue, which should be liberally applied. Wash in water and dry with filter paper. Put a drop of balsam about the center of the slip, which will be about the center and best part of the film; a clean cover will complete and make a permanent preparation. This method yields good slides of the blood as well as of the bacilli, showing the pathological changes that may take place in the blood and frequently white corpuscles engulfing the bacilli. The bacilli appear as short rods with square ends, which are a trifle thicker than the body. The ends also appear indented.

#### ASIATIC CHOLERA.

The bacillus of Asiatic cholera is found in the evacuations from the intestinal canal, and in most cases the disease may be recognized from preparations made direct from the evacuations as follows: Take a small portion of one of the slimy

flakes in the stool, spreading it with the platinum loop on a clean cover-glass held in a Cornet forceps. Dry in the air and pass rapidly through the flame of an alcohol or Bunsen burner three times. Apply a quantity of carbol fuchsin for about one minute. Wash in water, dry, and mount film side down in a drop of balsam on a clean slip. Stain another cover with Loeffler's alkaline-blue, and another with gentian-violet. If upon microscopical examination only curved rods are seen, their long axes extending in the same direction, the diagnosis of Asiatic cholera will be reasonably certain. If only a very few such rods are seen, a portion of the stool should be sent to the state or municipal laboratory with a history of the case for a bacteriological examination. A positive opinion is possible within twenty-four to thirty-six hours.

FIG. XI.—*Bacillus Asiatic cholera*. Stools. Stained with Loeffler's alkaline methylen-blue. Magnified 1200 diameters;  $\frac{1}{4}$ -inch oil immersion objective, Haensch & Lomb compensating ocular No. 2.

FIG. XII. *Bacillus mallei* Glanders, from nodule. Stained with gentian-violet. Magnified 1200 diameters;  $\frac{1}{4}$ -inch oil immersion objective, Zeiss projection ocular No. 4.

#### MALLEI—*Bacillus* of Glanders.

This disease, being a rather common one among horses and on account of its transmissibility to man and its not infrequent fatal termination, becomes an interesting study to the medical man. In horses the bacilli are found in the so-called glanders nodules in or upon the mucus membrane of the nose about the larynx, and not infrequently in the lungs. Deep ulcers develop from these nodules in the nose, especially about the turbinated bones. Microscopical examinations should be made from the ulcers and from the nodules, the latter being opened for this purpose. With the platinum loop take up some of the contents of a nodule and spread it on a clean cover-glass held in a Cornet forceps. Dry in the air, pass three times, film side up, through the flame of an alcohol lamp or Bunsen burner. Stain from one to five minutes with carbol fuchsin, methyl-violet, or Loeffler's alkaline methylen-blue. Wash in water, dry between filter paper, and mount, film side down, in a drop of balsam on a clean slip. The bacilli will appear as rods with rounded or slightly pointed ends. The bacilli stain irregularly, containing dark and light areas without any regularity whatever. The light spots are not spores.

In man the disease usually occurs in those handling diseased horses. It

may occur in the nose, but usually appears as abscesses, inflammations and necroses in the skin and muscles resembling carbuncles closely. When a positive diagnosis cannot be made from a cover-glass preparation, introduce into the peritoneal cavity of a male guinea pig a bit of the suspected tissue. If it is a case of glanders the testicles will begin to swell in thirty hours, the skin will become red and shiny, and pus will form, often breaking through the skin. This result is characteristic and positive of glanders.

Harvey Medical College.

WILLIAM H. KNAP.

## The Technique of Biological Projection and Anesthesia of Animals.

### II.

#### AVAILABLE LIGHTS AND THEIR LIMITATIONS.

In projection work of all kinds the most important factor is the light and its proper adjustment to the object. A safe rule is that the light should be stronger than is required for the magnification ordinarily used and capable of being developed at the will of the operator up to the maximum intensity needed for more difficult objects. Applying this rule to such sources of light as oil, gas with Welsbach burner, incandescent electric and acetylene, all of which are useful in producing certain grades of lantern slide pictures, it is evident that they can be used only with lantern slide projection lenses, and that they will fail to give satisfactory results with projection microscopes. Nevertheless, many instructive and interesting experiments upon small animals, measuring from one-quarter inch to several inches in length, are possible with the better lights of this class. With a one-fourth size projection lens, the magnification on the screen, placed at a distance of thirty feet from the lantern, is from fifty-five to sixty-five diameters. Small fish, especially goldfish, tadpoles, many aquatic larvæ, nymphs of dragon-fly, leeches, earthworms, small frogs and tree frogs, crayfish and similar types, may thus be made to exhibit their forms, motions, more or less of their internal organs, e. g., the alimentary canal, respiratory and circulatory systems, and even the circulation of the blood, if observed from near the screen. The methods of preparation of live animals, so that they may be used in the lantern in place of lantern slides, will be explained in a later article. The arrangement of light, condensers, projection lenses and screen is the same as for lantern slide work. Anesthesia with chlorotone is a valuable addition when the study of the circulation of the blood and internal organs is undertaken.

The lowest power artificial light which is strong enough to use with projection microscopes is oxyhydrogen. This gives far better results than the lights referred to above with all forms of work mentioned in the preceding paragraph, and is also suitable for microscopical projection of reasonably transparent objects up to a power of about one-thousand diameters. To reach this limit, however, careful attention must be paid to the adjustment of the jet and lime cylinder and their distance from the pair of condensers used for lantern slide work, also to a

third condenser, and in some cases to a substage condenser. The condensation of the light with its concomitant heat makes it necessary to introduce some form of heat screen between the pair of condensers and the microscope. The special feature of this apparatus is the third condenser, consisting of a plano-convex lens of about the same diameter as the condenser lenses used in the lantern, and of the same or longer focus. A little experience will show the need of such a lens, if theoretical considerations do not satisfy the mind. In adjusting the oxyhydrogen light for lantern slide work the aim is to secure maximum illumination of a surface three and a half inches or more in diameter with rays of light converging at such an angle that the entire cone of rays will enter the projection lens when at its correct focal distance from the slide. The aperture of a quarter size Darlot projection lens is about  $1\frac{5}{8}$  inch. If now an examination of this cone be made, it will be found that there is no sharply defined and very bright principal focus. In microscopical projection we have to do with fields ranging from  $\frac{3}{8}$  inch in diameter in a one-inch Wales projection objective to  $\frac{3}{16}$  inch in a No. 3 Leitz, about  $\frac{1}{5}$  inch in a No. 6 Leitz, and about  $\frac{1}{8}$  inch in a B. & L.  $\frac{1}{2}$  inch oil immersion. All the light which is to fall upon an area on the screen of from three to fifteen feet diameter, depending on the magnification, must pass through these small fields in the objects. The diameter of the front lens in a  $\frac{1}{2}$  inch oil immersion is only about  $\frac{1}{5}$  inch, and yet enough light must pass to give a bright picture covering a diameter of fourteen feet on the screen when a magnification of 9400 diameters is used. These considerations show the absolute importance of careful attention to the condensers. A word of caution is needed here, however, because of failures due to the use of condensers which give a pencil of rays having too wide an angle for the objective which receives them. In such cases the outer rays of the cone are not transmitted by the lenses of the objective, but are absorbed by the metal of the objective, with consequent danger from overheating the objective as well as imperfect illumination of the picture. Specific directions for adjusting the light and lenses when oxyhydrogen is used will be given when treating of the electric arc projection microscope.

The rapid development of electric light and power plants during the last few years has brought the most satisfactory artificial light within easy accuss of many thousands of people who have used, or wish to use, the projection microscope. With the electric arc light, and a suitable system of condensers, it is possible to obtain pictures on the screen with a magnification of from 5000 to 8000 diameters. The same rules in reference to condensers and heat screen apply as in using oxyhydrogen. Either direct or alternating currents may be used,—the objection to the latter being the continuous humming sound from the arc. A rheostat, suited to the voltage of the current, is used with the electric lantern.

We now come to the consideration of sunlight, the cheapest, as far as necessary apparatus is concerned, easiest to use, and by far the most powerful. All forms of projection work which can be done with the various artificial lights may be accomplished in a more satisfactory way with this natural light, and more than double the magnification is possible with high-power objectives. The author regularly uses a B. & L.  $\frac{1}{2}$  inch oil immersion lens which, with an amplifier, gives a magnification of 9400 diameters, and tests of similar lenses giving 10,000

diameters have been made without reaching the limit of the illuminating power afforded by an apparatus admitting a beam of sunlight four and one-half inches in diameter. When it is remembered that a magnification of 10,000 diameters means that the area of the picture on the screen is 100,000,000 times the area of the object, the enormous power of sunlight is still more evident. If it were not for its limitations, sunlight would be the ideal for all projection work, as it is, in fact, a necessity, for very high-power projection. A thin cirrus cloud or considerable smoke in the lower stratum of the atmosphere reduces the solar power to that of the electric arc; heavier smoke or thicker clouds reduces it through all degrees to below the minimum power for projection work. For about two hours after sunrise and the same time before sunset there is a marked reduction of power, varying with the condition of the atmosphere. Solar projecting apparatus should be placed in windows facing easterly, westerly, or preferably toward the south, where it will receive the unobstructed rays of the sun, thus limiting still further its availability. But in spite of all these limitations, the ease with which sunlight is used, its noiselessness, its steadiness when there is a clear sky, its great power and the small cost of necessary apparatus render it a very desirable light for projection work in school-rooms.

With a solar or electric arc projection microscope in a biological laboratory many difficult problems for beginners in microscopy are solved at once, the students' work is made more definite, the most typical slides prepared by the class or by the teacher may be exhibited for the benefit of all, and an endless variety of animal and plant species may be shown in such a way as to awaken interest, stimulate inquiry, and broaden knowledge gained by ordinary microscopical work, lectures, and reading.

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**A NEW PRESERVATIVE FLUID.**—In a recent number of the *Lancet* (161: 1334, 1335, Nov. 16, 1901) Galt gives the results of a series of experiments extending over several years, having as its object the discovery of a preservative fluid which would preserve the natural colors of anatomical specimens, and at the same time be free from some of the objectionable features of the well known Kaiserling's fluid. The fluid which was finally found to give the best results has the following formula:

Common salt	-	-	-	5 oz.	Chloral hydrate	-	-	-	1 oz.
Potassium nitrate	-	-	-	1 oz.	Water	-	-	-	100 oz.

Before being put into this solution for permanent preservation the tissues must undergo some preliminary treatment. The fresh tissue or specimen is washed for several hours in running water, and then fixed or "set" in an excess of methylated spirit. In case of large or soft specimens the addition of .5 per cent. of formalin accelerates the fixing of the tissues. The length of time which must be taken for this fixing process varies from six hours to a week according to the size and nature of the specimen. From this fixing bath the specimen is transferred directly to the preservative fluid. It may float in this for a short time before becoming thoroughly permeated. In some cases it was found necessary to renew the fluid after two or three weeks. After this no care is necessary except to replenish the jars with water, to make up for the loss by evaporation, in case the preparation is not sealed.

Advantages claimed by the author for this fluid are: 1. Its cheapness. 2. The simplicity of the process. 3. Specimens may be kept in the light without effect on the preservation of color. 4. Color preservation is better than in the older methods. 5. Its portability, requiring only the carrying of the solid ingredients. 6. It is non-volatile, non-poisonous, and non-irritating. 7. Its action on the cellular elements of the tissue is negative. 8. It causes only very slight shrinkage of the specimen.

RAYMOND PEARL.

## NOTES ON TECHNIQUE, III.

### *On Injecting the Blood Vessels of the Earthworm.*

In specimens of the earthworm prepared for dissection by preservation in alcohol, with or without previous treatment with chromic acid, the blood vessels are always found to be contracted and shrunken and not showing the characteristic color of the blood except in the largest vessels. Unless then an abundance of live worms are at hand which can be used for the study of the vascular system, class work on this particular subject is apt to be unsatisfactory. It is not usually convenient to furnish living material in quantity for the study of the circulatory system alone, and it has seemed highly desirable to make if possible injected preparations of the blood vessels so that students would be able to make out points which their own specimens did not show. After some experimenting on the subject it was found that good injections of the blood vessels of the common earthworm could be obtained. As the operation is in practice a rather difficult one, and since in the experience of the author it is impossible to attain successful results by the use of the ordinary injection methods, it seems advisable to publish an account of the procedure employed.



FIG. 1.—Showing form of the glass tube used for injection in place of syringe. About one-half natural size.

The main difficulties encountered were of two different sorts: (a) the smallness of the vessels made the introduction of the cannula very difficult, and (b) the walls of the vessels were so thin that they would withstand only the gentlest and steadiest of pressure. All attempts to inject by means of a syringe (hypodermic or larger) were unsuccessful. Furthermore, none of the masses ordinarily used for macroscopic injection nor any of several of the masses advocated for microscopic work were found to be adapted to this work. Either the mass would not run in the vessels or else it would cause them to burst, or finally it would not "set" after filling the vessels.

It was finally found best to use a bent glass tube drawn to a fine point at one end (Fig. 1) in place of a syringe. By blowing at the upper end of such a tube partially filled with injection mass it was found that a much steadier and more easily regulated pressure could be obtained than with any syringe. Furthermore, the tube could be better inserted into the vessels and held more steadily after

1 Tandler, J. Mikroskopische Injectionen mit kaltflüssiger Gelatin. Zeitschr. f. wiss. Mik. 18: 22-24, 1901. Reviewed in this journal, 5: 1625 and 1626.

It was found necessary to use a slightly larger amount of KI than is recommended by Tandler in order to keep the gelatine fluid until after injection. Eight to ten grams of the KI to 100 c. c. of fluid were used.

insertion than an ordinary cannula attached to a syringe. The injection which was found to give the best results was the cold gelatine mass recently devised by Tandler<sup>1</sup> for microscopic work. The mass was colored with a small amount of a saturated aqueous solution of anilin blue-black.

In detail the procedure was as follows: Large specimens of the common earthworm (*Lumbricus agricola*) were stupified in the ordinary way by the use of alcohol. They were not allowed to become entirely stupified, but just as soon as they had become sufficiently quiet they were opened with fine scissors along the dorsal side just to one side of the mid-dorsal line. The cut began just behind the clitellum and was carried forward in some cases clear to the anterior end, in other cases only to a point about two centimeters in front of the clitellum. After making this first cut the septa were cut down close to the body wall for a short distance only and the specimen stretched lengthwise and pinned out in a wax-bottomed dissecting pan in normal salt solution (.75 per cent. NaCl). No attempt should be made to dissect the septa down far enough to admit of pinning the specimen out flat, as it is almost impossible to avoid cutting some of the larger vessels. It was only found necessary to spread the worm enough so that the vessels on the intestine could be seen.

The glass tube was partially filled by suction with the injection mass, and its pointed end was inserted into the dorsal vessel, the point being directed forwards. It was found best to introduce the tube into the vessel at a point just under the clitellum, as here the dorsal vessel is considerably larger than at any other point in its course. It is necessary to get the tube into the vessel very soon after the worm is opened or it will not be possible to insert it at all, as the vessels contract strongly soon after they are exposed. It is not necessary to make any cut in the vessel for the insertion of the tube as the wall is so thin as to admit of its being pushed in directly. No ligature was used. After the tube is inserted, a steady, even pressure may be applied to the upper end of the tube by blowing, and in this way the mass may be started into the vessels. It was necessary to use a very gentle pressure at first, or otherwise the vessel would break immediately in front of the point of the tube. When once started the mass will usually run very freely and the pressure may be increased without rupturing the vessels. The injection should be continued till all visible vessels are distended. The tube may then be removed and the specimen put in 4 per cent. formalin for preservation and to harden the injection mass.

Specimens injected in this way have the dorsal vessel and all the vessels which come off from it in the region in front of the clitellum well filled. The parietal, intestinal and lateral œsophageal vessels fill completely and the excretory plexus shows very well. The "hearts" present a very striking appearance. For some reason which was not apparent the injection mass could not be made to pass from the "hearts" into the subintestinal vessel. Instead this vessel became distended with blood. No practical difficulty is caused by this lack of injection of the subintestinal vessel, as it is sufficiently large to be traced with ease in any ordinary specimen. By dissecting and pinning the specimens out in glass dishes containing a layer of black wax on the bottom, permanent preparations may be made.

RAYMOND PEARL.

## MICRO-CHEMICAL ANALYSIS.

## XIX.

## THE COMMON METALS.—SILVER GROUP.

III. *Arsenic Acid added to solutions containing Silver precipitates Silver Arsenate.*

*Method.*—Next to the test drop, which should be of moderate concentration, place a small drop of water into which introduce a little arsenic acid and then a tiny drop of ammonium hydroxide. Stir the reagent drop and cause it to flow into the test drop. A fine granular precipitate is immediately produced; later, thin plates and plate-like prisms appear (Fig. 78). The majority of the crystals which separate have the appearance of hexagonal plates. Their color by transmitted light varies from a reddish yellow in very thin plates to reddish brown with a tinge of dirty violet or even deep black as the thickness of the crystal increases.

*Remarks.*—The arsenic acid can be added directly to the test drop to either neutral or to weak nitric acid solutions, but the best and most uniform results seem to follow the procedure suggested above.

The amount of ammonium hydroxide added to the reagent drop must never be sufficient to neutralize all the arsenic acid and give rise to an alkaline solution.

Silver arsenate is insoluble in acetic acid, soluble in hot nitric acid and easily soluble in ammonium hydroxide. Good preparations can be obtained by recrystallizing from either of these solvents.

$\frac{1}{16}$  in.  $\times$  0.01 mm.  
FIG. 78.

In case ammonium hydroxide is employed, the colorless solution resulting contains the compound  $\text{Ag}_3\text{AsO}_4 \cdot 4\text{NH}_3$ , as has been shown by Widman. This tetra-ammonia salt can be made to crystallize in the absence of air in colorless needles, but on coming in contact with the oxygen of the air they turn red. It follows from this that the crystals obtained by recrystallizing silver arsenate from ammonium hydroxide are doubtless of variable composition.

Although the crystals of silver arsenate are neat, well formed and characteristic, the reaction cannot be considered as a satisfactory one for silver because of the fact that most of the other metals usually associated with silver are also precipitated by arsenic acid, thus seriously interfering with the test. Solution of the precipitated arsenate in ammonium hydroxide and drawing off, will usually affect a partial separation at least, and yield a more satisfactory test, but on the other hand the rendering of the drop alkaline may lead to the separation of arsenates which are soluble in acids but insoluble in alkaline solution.

It is of theoretical interest to consider in connection with the arsenic acid test for silver, the behavior of compounds of the elements analogous to arsenic as shown by their position in the Periodic System. We find, for example, crystalline salts of silver with phosphorus, as silver phosphate; with antimony, silver antimonate; with vanadium, silver vanadate; with chromium, silver chromates; with molybdenum, silver molybdates. Of these salts the chromates and vanadates can be employed for the detection of silver, but the phosphates, antimonates, and molybdates cannot be made to yield sufficiently characteristic results.

*Exercises for Practice.*

Test a neutral solution of  $\text{AgNO}_3$  in the manner suggested above.

Recrystallize a preparation of  $\text{Ag}_3\text{AsO}_4$  from  $\text{HNO}_3$ .

Try another preparation with  $\text{NH}_4\text{OH}$ .

Test a mixture of Ag and Pb. Then one of Ag and Hg.

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*With Sodium Phosphate.*

Neutral solutions of silver salts yield with this reagent an immediate dense yellow precipitate consisting of tiny grains. In a few seconds there appear stars, crosses and arrow-headed crystallites. Better crystals are obtained by recrystallizing from ammonium hydroxide. From this solvent dense, highly refractive yellow tetrahedra and globular masses separate, together with three and four armed crystallites which probably have the composition  $\text{Ag}_3\text{PO}_4$ .

In solution in ammonium hydroxide the salt seems to exist as the tetra-ammonia compound  $\text{Ag}_3\text{PO}_4 \cdot 4\text{NH}_3$  corresponding to that obtained with arsenic, but it is apparently less stable than the latter.

Silver phosphate can also be recrystallized from acetic acid and from nitric acid; the solubility of this salt in acetic acid being a distinction from the arsenate.

*With Oxalic Acid.*

Oxalic acid produces in neutral solutions an immediate fine granular precipitate of silver oxalate,  $\text{Ag}_2\text{C}_2\text{O}_4$ . After a few seconds thin plates appear which take the form of rhombs with very acute angles; hexagons and occasionally imperfect prisms are also seen. The rhombs extinguish parallel to a line bisecting the acute angles.

If, however, to the test drop there is first added a trace of nitric acid and then the drop of the reagent allowed to flow in, neat prisms are obtained. These prisms polarize strongly and show vivid colors between crossed nicols.

Often preparations of silver oxalate are obtained which bear a striking resemblance to crystals of cadmium oxalate.

*Tartrates of Silver.*

Primary sodium tartrate added to test drops just acidified with nitric acid precipitates clusters and single prisms with obliquely truncated ends. Twins are frequent. (The preparations obtained resemble closely those of the double oxalate of potassium and glucinum, Fig. 65.)

This silver salt seems to exhibit oblique extinction, probably belongs to the monoclinic system, and may have the formula  $\text{HAgC}_4\text{H}_4\text{O}_6 \cdot 5\text{H}_2\text{O}$ . The normal tartrate  $\text{Ag}_2\text{C}_4\text{H}_4\text{O}_6$  has been prepared by precipitating silver nitrate with tartaric acid. It is therefore probable that the composition of the precipitate obtained in "Micro" testing will vary according to the reagent used, i. e., tartaric acid, or primary sodium tartrate, or a mixture of the two.

Potassium antimonyl tartrate precipitates the compound  $\text{Ag}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \text{H}_2\text{O}$ , at first as an amorphous mass, soon crystallizing in tufts of radiating needles. If the preparation be heated, the precipitate dissolves and on cooling beautiful highly refractive triclinic rhombs separate. It is not always an easy matter to obtain just the right conditions leading to the formation of good crystals.

(See in this connection Barium VI, JOURNAL, 4: 1827-28.)

*With Potassium Antimonate.*

This reagent applied to neutral solutions in the manner suggested under Sodium (q. v.) yields an amorphous precipitate soon crystallizing in long acicular prisms with bristly edges and showing a decided dendritic tendency. These long needle-like crystals often disintegrate rapidly, leaving only a mass of irregular fragments. Tiny crystallites are also seen in the form of stars and crosses. Occasionally lenticular grains singly and in masses appear.

This reaction of silver with potassium antimonate is a most instructive one if we recall the fact that in the Periodic System silver falls in Group I under sodium, and should, therefore, behave toward some reagents in a manner analogous to the latter. As a further analogy between silver and sodium we have already seen that the normal anhydrous sulphates of the two elements are isomorphous.

*Silver Acetate.*

This salt separates from concentrated solutions upon the addition of acetic acid or alkaline acetates, first as a mass of thin plates and thin needles and prisms; soon large, thin, colorless striated irregular plates are formed. These plates, though irregular, still retain the general outline and proportions of the more perfect plate-like prisms from which they are developed. They extinguish parallel to their length.

This reaction is an important one, since silver acetate may separate whenever silver is present and an alkaline acetate is added to mitigate the action of a mineral acid.

*With Ammonium Carbonate.*

Silver carbonate is first precipitated in a fine granular condition; later, neat, highly refractive crystals of cuboidal aspect, and spherical masses, appear. The reaction is an interesting one and the preparations obtained well worth examination and study.

Like the precipitation of a number of other silver salts, this reaction is of more importance with reference to the possibility of its interfering with, or obscuring, certain reactions for other elements.

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## A Review of the Existing Methods of Cultivating Anaerobic Bacteria.

### I I.

#### REPLACEMENT OF AIR BY INERT GASES.

This principle has been employed by *Pasteur* in the study of his *Vibrio butyricus* as early as 1861. He cultivated this organism in an atmosphere of hydrogen and carbonic acid. Since then many methods of this type have been introduced and put in practice. Of the various gases that have found application as a means of replacing the air, hydrogen proved to be, everything considered, the most satisfactory.

Hydrogen is produced most conveniently by means of the Kipp Generator, dilute hydrochloric acid and metallic zinc being used. The hydrogen thus produced is freed from traces of  $\text{AsH}_3$ ,  $\text{SH}_3$  and  $\text{PH}_3$  that may be present, by passing the gas through concentrated  $\text{KMnO}_4$ , from acids that may have been carried over from the generator by passing it through concentrated  $\text{KOH}$ , from traces of oxygen by passing it through an alkaline solution of pyrogallol, and from water by passing it through dry  $\text{CaCl}_2$  or concentrated  $\text{H}_2\text{SO}_4$ . If chemically pure zinc is used, washing is not necessary.

When a union is to be made between two glass tubes by means of rubber tubing the ends of the glass tubes should meet. This avoids the direct exposure of large surfaces of rubber tubing to the action of hydrogen and prevents the diffusion of hydrogen through the porous walls of the rubber tubing. In any case it is advisable to vaselinate the rubber tubing.

If the apparatus is sealed by means of glass turn-cocks, only cocks with diagonal openings should be used. They afford a perfect seal.

High pressure in the apparatus tends to seriously affect the cultures and should therefore be avoided. Slight over-pressure which will not materially disturb growth is desirable as a means of preventing any possible diffusion of gases. Where convenient, it is well, instead of filling the apparatus with hydrogen and sealing it hermetically, to pass the hydrogen through the apparatus continually during the whole period of cultivation. In this case the hydrogen, upon leaving the culture apparatus, is conducted through a doubly perforated rubber stopper to the bottom of a wash bottle containing distilled water. The water forms a hermetical seal, preventing the air from entering the apparatus in case the gas pressure should diminish.

The purity of the gas in the culture apparatus is tested by filling a test tube with the escaping hydrogen and applying a match to it. If the gas burns with explosion, the apparatus still contains some atmospheric oxygen, a quiet flame indicates pure or nearly pure hydrogen.

#### A. CULTURES IN TUBES AND FLASKS.

*Hauser* and *Liborius* (1886) introduced the apparatus shown in Fig. 8. A short distance above the surface of the medium the test tube carries a lateral

tube, constricted near its union with the test tube, and cotton-plugged at its outer opening. The test tube is also constricted below the lower end of the cotton plug.

*Method.*—Introduce the medium into the test tube by means of the drawn-out funnel (f), replace the cotton plug and sterilize as usual. Inoculate the medium and connect the end (b) of the lateral tube with the Kipp generator. Pass gas through for fifteen to thirty minutes. In case gelatin or agar are used stand the test tube in a water bath at 40°C. while the gas is introduced. Now seal first the test tube and then the lateral at their respective constrictions in the flame. Similar apparatus have been constructed by Exner, Buchner and Roux. For agar, blood serum, and potato slant cultures Liborius arranges the medium so that the slanted surface is opposite the lateral tube.

FIG. 8.

*Hueppe* closes the test tube or flask (Fig. 9) containing the inoculated medium with a doubly perforated rubber stopper. In one perforation rests a glass tube (L) which reaches to the bottom of the flask, the other holds the glass tube (A) containing at its lower curve a small amount of mercury.

*Method.*—Introduce hydrogen at (L); the air is forced out through (A). Having passed hydrogen through the tube for from ten to twenty minutes, seal the glass tube (L) in the flame. The mercury in tube A serves as indicator of the changes in pressure, which may take place as the result of gas production by the growing bacteria in the tube or flask. If this indicator is not desired, a short glass tube is used in the place of tube (A) and when all the air is replaced by hydrogen the glass tube is sealed in the flame.

*Fraenkel's* method (1888): Into a test tube (Fig. 10) containing liquified inoculated gelatin or agar, insert a doubly perforated well fitting rubber stopper carrying two glass tubes; one reaching to the bottom of the test tube, the other to the lower surface of the stopper. Cover the top of the rubber stopper and test tube with an air-tight layer of paraffin or sealing wax. Introduce hydrogen through the long glass tube. When all the air is replaced by the gas, seal first the exit and then the entrance tube over the flame, and roll the test tube until the medium is congealed.

For agar stick and stab cultures, *Blucher* (1890) recommends the following method:

FIG. 9.

FIG. 10.

Reverse the inoculated test tube (see Fig. 11), remove the cotton plug; with the open end downward dip the test tube into a beaker containing a solution of diluted glycerin (equal parts glycerin and water). By means of glass tube (g) introduce hydrogen into the test tube. In about five minutes all the air is replaced by the gas, the generator is disconnected, and the beaker containing the test tube is put into the incubator.

*Hesse* (1890) modified *Blucher's* methods by inverting the inoculated test tube into mercury instead of glycerin.

*Fuchs* (1890) rejects the condensation water from slanted blood serum tubes, inoculates the slanted surface, reverses the tube and introduces hydrogen for about five minutes. Without changing the position of the test tube he then inserts a tightly fitting, sterilized rubber stopper and seals hermetically by dipping the sealed part of the tube into liquid paraffin.

FIG. 11.

*Ogata* (1892) used a method very similar to that of *Liborius*. Instead of introducing the gas through the lateral tube as *Liborius* did, *Ogata* does away with the lateral tube and conducts the gas down into the medium by means of a capillary glass tube running through the cotton plug of the test tube down to the bottom of it (see Fig. 12). The air is slowly forced out of the tube in form of gas-bubbles, some of which collapse, others form foam. As soon as the foam has passed up through the narrow part of the tube, the capillary tube is removed and while there is still foam in the upper portion of the tube the constriction is sealed over the flame.

*Heim* (1892), who claims to be the inventor of this method, recommends that, except when *Esmarch* roll cultures are made, the test tube should be sealed without removing the capillary tube; that the inoculation should be made before the tube is constricted, and that the constriction should not become wet, as the glass is liable to crack during the operation of sealing in the flame.

For liquid medium *Roth* (1893) used an upright flask (see Fig. 13). In the lower part of the neck rests a cotton plug, which is attached to a wire running out through the flask. Through the cotton plug passes one end of a glass tube, the other being conducted into a cup of glycerin.

*Method.*—Fill the flask about half full with liquid medium, press the cotton plug (c) well down into the neck, push the glass tube down near the bottom of the flask and connect the other end of it with the gas generator. When all the air is replaced by the gas, and before disconnecting the apparatus from the generator, raise the glass tube out of the medium as shown in Fig. 13, immerse the other end of the glass tube in glycerin, fill the neck above the cotton

FIG. 12.

plug with paraffin and disconnect at (e). The flask is opened by heating the neck in the flame and raising the plug by means of the attached wire. In case of very large flasks Roth covers the cotton plug with a piece of rubber containing an opening for the introduction of the glass tube before the apparatus is connected with the generator. This will increase the pressure from within and prevent any possible entrance of air during the introduction of gas.

Novy (1893) devised an apparatus which allows a large number of tube cultures to be made simultaneously. The apparatus as shown in Fig. 14 consists of a cylinder 20 x 10 cm. (not counting the neck). The neck carries two lateral tubes. Into the neck of

FIG. 18.

the cylinder is fitted a glass stopper with ground surface. The glass stopper also carries two perforations on opposite sides corresponding to those in the neck. From the inside of the glass stopper one of the perforations is connected with a glass tube, reaching nearly to the bottom of the cylinder. If a gas heavier than the air is introduced, the glass stopper should be turned so as to conduct the gas through the tube in the interior to the bottom of the cylinder, thus allowing air to escape on top through the other lateral tube. In case of a gas lighter than air, as for instance hydrogen, it should enter the cylinder on top, forcing the air out through the long tube reaching to the bottom of the cylinder.

*Method.*—Inoculate forty to fifty ordinary culture tubes (12 to 15 cm. long). Insert loose cotton plugs and cut them off at the end of the tubes. With a pair of long tongs place the tubes in the cylinder, the bottom of which is covered with cotton, cover the surface of the glass stopper with paraffin or vaseline and insert the latter in its place, care being taken that the perforations in the stopper correspond with those in the neck of the cylinder. Connect the apparatus, as above directed, with the gas generator and lead the exit tube into a wash bottle containing water. After passing the gas through for from one to two hours, carefully turn the glass stopper an angle of 90°, disconnect the cylinder and put it aside for the development of the bacteria. If instead of replacement by gas the cylinder is evacuated, it becomes impossible to turn the glass stopper. In this case each lateral tube carries a small glass turn-cock for

FIG. 14.

the purpose of sealing the apparatus when exhaustion is complete.

*Hewett* (1894) recommends for bouillon cultures the use of an yeast flask of 90 c.c. capacity. The flask is closed by a monopерforated, well fitting rubber stopper through which a glass tube passes to the bottom of the flask. The part of the tube extending above the stopper is cotton plugged. A lateral tube projects from the side of the neck as shown in Fig. 15. The lateral tube is also plugged with absorbent cotton. It leads into a cup containing mercury, the latter forming a valve. The surrounding air cannot enter, while the interior air and the gases formed by bacterial activity have free exit.

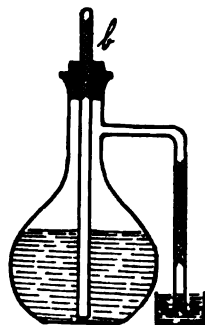


FIG. 15.

*Method.*—Fill the flask about two-thirds full with glucose bouillon, sterilize, cool and inoculate the medium. Introduce hydrogen through the glass tube in the rubber stopper for one hour, before disconnecting the generator dip the end of the lateral tube into the cup containing mercury, and seal tube (b) above the rubber stopper in the flame.

*Lubinski* (1894) constructed two forms of apparatus. One of these resembles that of Novy so closely that it need not be described here. The second apparatus is illustrated in Fig. 16. It is closed with a ground glass stopper. Immediately below the neck the cylinder carries at two diametrically opposed places tubes (t) and (t<sub>1</sub>), ending in glass bulbs. Both bulbs are partly filled with liquid paraffin or vaseline. In order to prevent the liquid in bulb (t) from passing over into the apparatus, bulb (t) is separated from the cylinder by a second bulb. The gas is introduced through bulb (t<sub>1</sub>); bulb (t) serves as exit for the air. As in Novy's apparatus the entrance of gas and exit of air take place at different heights. The manipulation is very similar to that of Novy.

*Jacobits* (1901) successfully made agar slant cultures in nitrogen atmosphere. He used Fraenkel's tube. Into the boiling hot agar he introduced a current of nitrogen purified by running through concentrated sulphuric acid, through alkaline pyrogallol and through potassium hydroxide. He then lays the tube on an ice tray to slant the agar, continuing the current of nitrogen until the agar is congealed. After inoculating in the usual way more nitrogen is introduced. Finally the entrance and exit gas tubes are sealed hermetically in the flame.

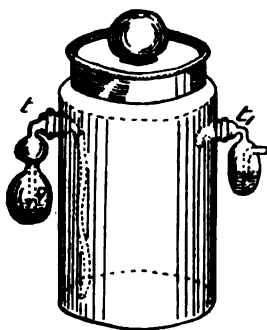


FIG. 16.

## B. PLATE CULTURES.

*Kitasato* (1889) used a flattened receptacle about 2 cm. thick (see Fig. 17) for the isolation of tetanus. The manipulations of the apparatus resemble those employed in the case of Liborius tubes. The two openings are plugged with cotton and the apparatus is sterilized. Then the surface tube (b) is constricted in the middle. With a well drawn out funnel about 20 c. c. of the liquified inoculated medium are poured



FIG. 17.

through the opening at (a) into the receptacle, then (a) is also constricted. Having passed hydrogen through until all the air has escaped, the two ends, first the exit, then the entrance, are sealed in the flame.

*Roth* (1893) recommended a similar device. His apparatus is illustrated in Fig. 18. It is used for solid medium.

*Method.*—Plug both openings with absorbent cotton and sterilize. The plug at (a) carries a corkscrew, that at (b) is attached to a fine copper wire. Introduce about 8 c. c. of the liquified medium (gelatin or agar) and sterilize on three successive days. For this purpose the flasks may be stood upright in wire baskets. After inoculating in the usual way, let the medium congeal. Then push by means of the cork screw the plug at (a) down far enough to touch the medium; introduce hydrogen at (b) by connecting (b) by means of rubber tubing with a generator. The rubber tubing carries a clamp. In order to prevent the mixing of air and gas as much as possible, it is best to incline the apparatus so that the neck points downward. When all the air is replaced by the inert gas pour a little melted paraffin on the cotton plug in the neck. When congealed dip tube (b) into liquid paraffin and remove the rubber tubing. The plug in tube (b) is thus saturated and the tube filled with paraffin. This paraffin seal proved very satisfactory. In order to get access to the grown colonies, warm the neck and pull the cotton plug out by means of the corkscrew.

For making cultures in the field *Roth* used a similar apparatus (see Fig. 19). In order to avoid breakage, the small tube (b), shown in Fig. 18, is discarded and the hydrogen is introduced in the laboratory. For this purpose a small cotton plugged sterile metal tube is inserted in the apparatus. When the air is all driven out, the neck is filled with paraffin and the metal tube carefully removed by means of a copper wire which had previously been attached to it.

*Blucher* (1890) recommends the apparatus illustrated in Fig. 20. A funnel shaped bell jar with a cotton plugged opening (D) and weighted down with lead (F) rests in a glass bowl (A). The petri dish is kept in its place by means of a spring wire ring with three projections reaching to the walls of the bowl.

*Method.*—Pour the inoculated medium into the open petri dish (B). Place the bell jar over it and pour into the glass bowl diluted glycerin (1 part glycerin, 3 parts water) until the interior is completely separated from the exterior. Introduce hydrogen through the opening at (D). The air escapes through the glycerin in bubbles. When all the air is out, generally after



FIG. 18.

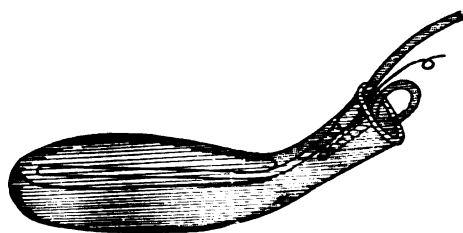


FIG. 19.

about 10 minutes, close the rubber tubing at (D) by means of a clamp, cut the tubing about two cm. above the clamp and fill the end with glycerin. The freedom of the apparatus from oxygen can be tested as follows: Bring a burning match close to the glycerin in the bowl where the bubbles escape. If the latter burn regularly and without explosion, the apparatus may be considered oxygen-free. In order to obtain access to the petri dish when the culture has developed, carefully and slowly raise the bell jar on one side, allowing small bubbles of air to enter. This will prevent the glycerin from spattering into the culture.

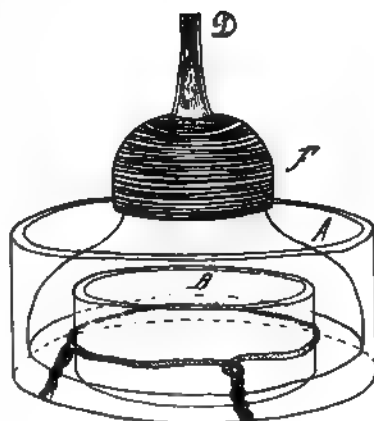


FIG. 20

*Botkin's apparatus* (Fig. 21) is a modification of that of Blucher. It contains a glass dish (D) 20 to 25 cm. in diameter (much the same as those used for potato cultures). In the dish (D) stands a wire support for the petri dishes, which are covered by bell jar (B). The latter has a diameter about three cm. smaller than dish (D). It does not touch the bottom of (D) directly, but it rests on a cross band of lead (L) one cm. in thickness. U tube (U) is a thin rubber tube; its lumen contains a fine, soft, and flexible copper wire. Opposite tube (U) there is another similarly constructed rubber tube (F) leading from the

u  
D

FIG. 21.

interior of the bell jar into a wash bottle (W) containing water and closed by a doubly perforated rubber stopper; the second perforation carries a glass tube continuing into a rubber tube and closed by a clamp (C).

*Method.*—Disinfect the interior of the apparatus by washing with a solution of sublimate and drying with alcohol and ether. Sterilize the wire support in the flame. Prepare the culture plates in the ordinary way and place them on the wire stand. Pour a layer, three cm. high, of paraffin liquidum, preferably Buchner's mixture (1 part glycerin to 3 parts water) into dish (D). Insert the U tubes

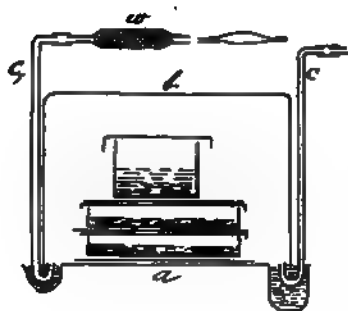


FIG. 22.

in their respective places and cover with the bell jar. Introduce hydrogen through tube (U). The air escapes in bubbles through the glycerin in dish (D). In about ten minutes open clamp (C), allowing the air in wash bottle (F) to escape. After two more minutes light the gas escaping at (C). If the apparatus contains pure hydrogen the escaping gas will burn with a quiet, even flame, otherwise with a crackling noise. Being assured of the complete replacement of air by hydrogen, carefully withdraw the U tubes (U) and (F)

from the apparatus. In order not to disturb the glycerin seal by transportation, Botkin recommends placing the apparatus in the incubator before hydrogen is introduced.

Hesse (1892) introduced the type of apparatus shown in Fig. 22. It consists of the following parts:

a. A cast iron plate 20 cm. in diameter, with a channel (2 cm. wide and 3 cm. deep) at its periphery; on one side the channel is  $2\frac{1}{2}$  cm. deeper than at the other. It is filled with mercury. The plate is smeared with shellac.

b. A bell jar fitting into the channel and floating on the mercury.

c. Two U tubes (c) and (c<sub>1</sub>), with extensions for the entrance and exit of gas and air respectively. Tube (c<sub>1</sub>) contains at (w) a wire gauze to ensure a safe test of the escaping gas by burning.

*Method.*—Cover the center part of plate (a) with a blotting paper for the purpose of absorbing moisture. Upon this place the inoculated, loosely covered plate cultures, invert the bell jar over them and insert the U tubes (c) and (c<sub>1</sub>) in their proper places. Connect (c) with the Kipp generator. The purity of the escaping gas is tested by applying a burning match to the capillary end of the tube (c<sub>1</sub>).

Baginsky constructed an apparatus (Fig. 28) that appears to be simple in construction and easy to manipulate. It consists of a large metal plate, the circumference of which is covered with a thick rubber ring. A bell jar is inverted over the plate and rests on the layer of rubber. Over the bell jar is placed a metal plate similar to that which forms the bottom of the apparatus. The bottom part contains four projections in which are hinged metal rods, the outer ends of these rods fit into similar projections in the cover plate. By means of these four metal

FIG. 28.

rods the upper and the lower metal plates are tightly pressed against the bell jar, closing the apparatus hermetically. On opposite sides the bell jar contains small lateral tubes by means of which hydrogen is introduced and air driven out.

*Method.*—Place the inoculated petri dishes upon the bottom plate of the apparatus. Invert the bell jar and seal the apparatus by screwing the upper and lower plates firmly against the bell jar. Introduce hydrogen at the upper lateral tube, the air will escape through the other. When all the air is driven out, which is determined by the hydrogen tube test, seal first the exit, then the entrance of gas and place the apparatus in the incubator. A very satisfactory way is also to run the hydrogen through continuously until the cultures are grown.

This apparatus is also well suited for a large number of tube cultures in hydrogen atmosphere.

*Novy* (1893) recommends his apparatus (Fig. 6), designed for plate cultures in a vacuum, also for plate cultures in hydrogen atmosphere. In this case hydrogen is introduced at one end of the glass cock (x-y) and the air escapes at the other.

FIG. 24.

In addition he modified his apparatus for tube cultures (Fig. 14) so that plates can be placed in it (see Fig. 24). Its manipulation is the same as that for the tube cultures.

*Kedrowski's* apparatus (Fig. 25) consists of a deep glass plate (C) with cover (D). On the sides at diametrically opposite points plate and cover are perforated for the entrance of gas and the exit of air.

*Method.*—Into the sterile plate (C) put an open petri dish containing the inoculated medium. Coat the inside of the rim of cover (D) with vaseline. Place D over C, so that the perforations in plate and cover meet perfectly. Introduce hydrogen, and when the gas has replaced all the air, then turn the cover 90° and put the apparatus into the incubator.

*Gabritschewsky's* plate (Fig. 26) consists of a sub-plate with a rim at its periphery. The outer end of the rim is so constructed as to expose a broad horizontal surface for the cover to rest on. This surface is perforated at two diametrically opposite points corresponding to two similar perforations in the cover.

*Method.*—Pour the inoculated medium into the central part (c) of the sub-plate. Cover with a paraffined ground glass cover so that the holes in the rim cor-

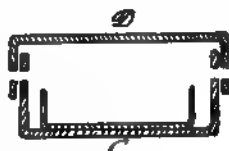


FIG. 25.

respond with those in the cover. Introduce hydrogen and when all the air is replaced, carefully turn the cover 90 degrees, sealing hermetically. In addition



FIG. 26.

Gabritschewsky recommends the use of an alkaline solution of pyrogallol which is poured into the rim before the gas is introduced.

A similar plate (Fig. 27) was constructed by Kamen. Its method of manipulation is

exactly the same as that of Gabritschewsky.

In case of *Beck's* plate (Fig. 28) a common petri dish is used. It is covered with a plate carrying two lateral tubes for the introduction of gas and exit of air. The periphery of the cover is so shaped as to form a small reservoir which may be filled with water in case of cultures that require a long period of incubation. By means of short pieces of stout rubber tubing the laterals are connected with short constricted glass tubes which are sealed in the flame when the plate is oxygen free.

*Method.*—Pour the inoculated medium into the sterile plate and put the cover in its place. When the medium is congealed fill the rim (X) between

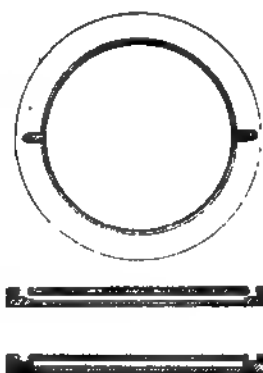


FIG. 27.



FIG. 28.

FIG. 28.

the plate and the cover with liquified paraffin. When this is solidified replace the air by gas and seal the constricted tubes in the flame.

*Aren's* plate (Fig. 29) consists of an ordinary petri dish carrying lateral tubes which open into the interior of the plate. The space between the rim of the cover and that of the plate is filled with liquified paraffin. When the air is expelled by gas the laterals are sealed in the flame.

*Epstein's* plate is a modification of that of Aren. Instead of the lateral tubes of glass similar tubes of firm rubber are used. The rubber tubes are a part of a solid rubber band that covers the periphery of the plate. When the hydrogen has replaced the air the plate is sealed by pushing well fitting glass rods into the lateral rubber tubes.

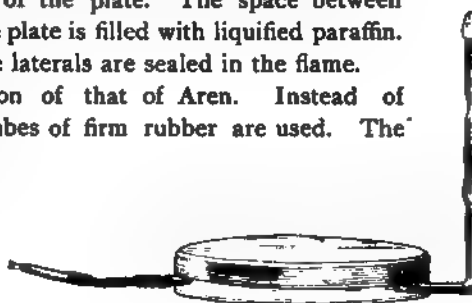


FIG. 29.

## III.

## ABSORPTION OF ATMOSPHERIC OXYGEN.

To this category belong all those methods, in which advantage is taken of the oxygen-absorbing power of some chemical agents. The adoption of this principle for the purpose of bringing about anaërobic conditions is of more recent date than that of exhaustion and of replacement by inert gases.

In 1878 *Gunning* used a mixture of a ferro salt with an excess of sodium hydroxide. He also recommended a solution of glucose containing indigo carmine and sodium hydroxid.

In 1880 *Nenki* found that anaerobic bacteria grow well in an atmosphere from which the oxygen has been absorbed by an alkaline solution of pyrogallic acid. Practical application of this principle, however, was not made until 1888, when *Buchner* invented the method which now carries his name.

*Buchner's Method.*—Use two test tubes of different sizes (see Fig. 80), drop into the large tube a small wire support on which the small tube is subsequently placed. The small test tube contains the inoculated medium and is closed with a loose cotton plug. Put into the large test tube one gram of dry pyrogallic acid and then ten c. c. of a one-tenth solution of potassium hydroxid (1 part Liquor Kali caust. to 10 parts of water). Then quickly lower the small culture tube and close the latter hermetically with a new, elastic, well fitting, paraffined rubber stopper. Shake well. *Buchner* observed that the above stated amount of pyrogallic acid and potassium hydroxid will completely absorb the oxygen in a tube with a cubic content of 100 c. c. in 24 hours at incubator temperature. At lower temperatures, as for instance in the refrigerator, the absorption of oxygen is much slower. Frequent shaking hastens the absorption.

FIG. 80.

For plate cultures *Buchner* incloses the plates filled with the inoculated medium under a hermetically sealed bell jar and uses larger amounts of pyrogallic acid and potassium hydroxid.

*Babes* and *Puscarin* in 1890 modified *Buchner's* method by putting the inoculated culture tubes into a *Fresenius* desiccator containing a large amount of pyrogallic acid and potassium hydroxid. After closing the desiccator hermetically with a vaselined ground glass plate, the apparatus is put into the incubator. This method enables the experimenter to cultivate numerous tube cultures simultaneously and under identical conditions. The authors obtained the best results when they used media containing 2 per cent. glucose.

*Blucher* (1890) recommends the following method for plate cultures:

Put a petri dish 6 cm. in diameter, minus cover, into a glass saucer 10 cm. in diameter and 4 cm. high. The upper edge of the saucer is ground convexly and it fits exactly into a cover with a concave rim. Into the outer saucer pyrogallic acid and potassium hydroxid are placed and the cover, the concave panel of which has been well vaselinated, is inverted over the saucer. The decrease

of pressure within, due to the absorption of the oxygen in the apparatus, causes the over-pressure from without to force the cover tightly over the saucer, forming a hermetical seal. In order to reopen the apparatus the cover must be turned before it can be taken off.

*Trambusti's Method.*—The apparatus used by this investigator is illustrated in Fig. 31. It consists of two parts: a cone-shaped flask (A) containing the medium, and a cylinder (B) which is screwed into (A) by means of a fine thread. In the interior of (B) there is a small tube which opens into (A).

FIG. 31.

*Method.*—Pour the inoculated medium, gelatin or agar, into flask (A) and distribute it over the surface of the bottom uniformly, the same as in case of a petri dish. Screw (B) into (A), remove the stopper and fill (B) with an alkaline solution of pyrogallol (2 grams of dry pyrogallol plus 15 c. c. of 10 per cent. solution of KOH), care being taken that

the reagent does not enter the small tube. Replace the stopper which has previously been covered with paraffin or vaseline and remove the apparatus to the incubator.

*Arens* in 1894 used a common small desiccator with a ground glass cover for anaërobic plate cultures.

*Method.*—Fill the desiccator partly with quartz sand, to this add an optional amount of dry pyrogallol acid, leaving just room for one or two small petri dishes. Use deep layer of agar in the plates. Before putting the plates into the apparatus pour a considerable amount of a 10 per cent. solution of caustic potash all over the surface of the sand and acid. Then place the petri dish, minus cover, upon the sand and close the desiccator with a well vaselined ground glass cover by rotary motion.

*Lubinsky* in 1894 modified Buchner's method for tube cultures. Instead of putting the culture tube into a large test tube, Lubinsky placed it in a glass cylinder (Fig. 32) 12–15 cm. high and 3–4 cm. in diameter. Into this cylinder he pours the alkaline solution of pyrogallol acid; then he pushes a snugly fitting, manifold perforated cork about half way down into the cylinder. In the center of the cork there

FIG. 32.

is a large perforation in which the culture tube is inserted. The cylinder is closed with a glass stopper of ground glass and the edges are sealed with paraffin. When sealed he shakes the apparatus vigorously for 2 to 3 minutes to hasten the process of absorption.

*Novy* recommended his apparatus (Fig. 24) designed for cultivating anaërobes in a hydrogen atmosphere, also for the pyrogallol method.

*Ucke* (1898) manipulated *Novy's* apparatus (Fig. 24) as follows: Put 10 grams of dry pyrogallol into a small beaker, float this beaker in a larger one (120 c. c. capacity) containing 50 c. c. of a 20 per cent. solution of potassium hydroxid and stand the two beakers in the apparatus, so that the glass tube reaching from the glass stopper nearly to the bottom extends into the small beaker. At the last moment, when the apparatus is ready to be put into the incubator, introduce 50 c. c. of water through the long glass tube and turn the glass stopper 90°. This causes the small beaker to sink and the solution of potassium hydroxid mixes with the pyrogallol. By this manipulation the pyrogallol does not get access to the alkali until the apparatus is sealed up, hence it is a means of economizing the oxygen absorbing power of the pyrogallol.

*Wright* (1901) recommends the following modification: Into a medium-sized test tube (see Fig. 33) containing the inoculated culture medium, push a sterile cotton plug well down. By means of a pipette pour  $\frac{1}{2}$  c. c. of a saturated solution of pyrogallol and 1 c. c. of a 50 per cent. solution of sodium hydroxid upon the cotton plug. Seal the tube with a well fitting rubber stopper with as little delay as possible. The quantity of the reagents added is so small that there is no danger of its running down along the side of the tube into the culture medium. According to *Wright* this method can be used successfully for all kinds of flasks and tubes. He lays stress on the fact that the nutrient medium should be freshly boiled and of alkaline reaction.

FIG. 33.

*Slupski* (1901) uses the following modification for plate cultures: His apparatus (Fig. 34) consists of a bell jar 15 cm. in diameter and 5 cm. high, ending at the bottom in a ground glass rim  $1\frac{1}{2}$  cm. in width. The bell jar is placed in a glass dish about 10 cm. high. On the bottom of this glass dish rests a double plate. The outer plate (b) contains water and the inner (a) is designed for pyrogallol. Over the double plate rests a tripod which supports the open petri dish.

*Method.*—Pour the inoculated culture medium into the open petri dish. Fill plate (b) with water and heap plate (a) with dry pyrogallol (25 grams). To this add 50 c. c. of warm distilled water. Replace tripod. Throw two pieces of KOH (14 gms.) into the pyrogallol. Quickly put a blotter and black paper on top of the tripod, upon which place the open petri dish, invert bell jar (X) into glass dish (Y) and seal with a thin layer of paraffin. Then pour hot paraffin three to four cm. deep into dish (Y) and when cooled and congealed pour some liquid paraffin on top. Place the apparatus for about 50 hours in the refrigerator at a temperature of 5–6°C. in order to prevent any growth while the absorption of oxygen is still in process. Then put it in the incubator.

*Hammerl* (1901) uses a mat of heavy cardboard, felt, or cellulose which he

saturates with a concentrated solution of pyrogalllic acid. This absorbing material he fits into the cover of a deep petri dish.

*Method.*—Weigh 4–5 gms. of dry pyrogalllic acid into a small beaker, add a 50 per cent. solution of potassium hydroxid, drop by drop, and shake until the

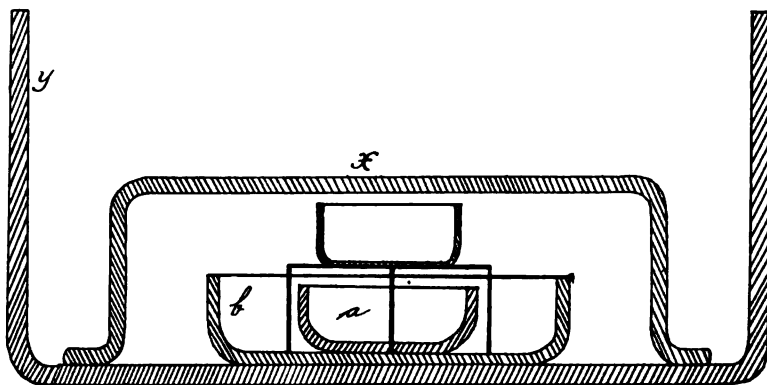


FIG. 34.

pyrogallol is perfectly wet. In a few minutes it will be dissolved. The less potassium hydroxid used the greater is the absorbing power of the solution. Drop the solution of alkaline pyrogallol upon the mat of cardboard, felt or cellulose. Do not saturate the absorbing material completely, otherwise some of the reagent might run down into the medium. Invert the cover containing the absorbed reagent over the culture plate, which has previously been filled with the inoculated medium. Seal the space between the rim of the cover and that of the plate with a mixture of white wax (12 parts) and beef tallow (100 parts) and finally cover the seal with a firm and well fitting rubber band.

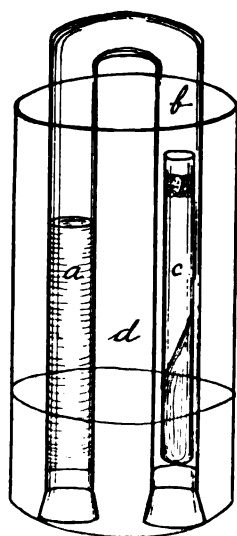


FIG. 35.

For single tube cultures *Smith\** uses the following device: Into one end of a large U tube insert a small test tube containing the inoculated culture medium. Invert the other end over a small slender flask or vial containing a strongly alkaline solution of pyrogallol and standing the ends of the tube in a dish containing oil.

The writer modifies Smith's method as shown in Fig. 35. Into one end of a large U tube place about three grams of dry pyrogalllic acid and three grams of sodium hydrate. Close this end with a rubber stopper and pour about 15 c. c. of water into the other end (b) holding the U tube so that the water all escapes into the branch (a) containing the reagents. Now insert in the second branch (b) a small test tube (c) containing the inoculated medium and a loose cotton plug. Close this end of the U tube with a rubber stopper and stand the U tube in a beaker (d) containing mercury or glycerin. Good results were obtained by this method.

\*The method of Dr. Erwin F. Smith, Department of Agriculture, Bureau of Plant Industry, Washington, D. C., has not as yet been published.

*Nikiforoff* introduced a method by which anaërobes can be cultivated in a hanging drop.

*Method.*—Use a slide which carries a ground glass ring, just as for making an ordinary hanging drop preparation. Cover the ground edge of the ring which is used for the support of the cover glass with vaseline. Place the cover glass with the hanging drop on the ring, so that one side of the ring is not covered. Insert here a small drop of pyrogallic acid. Move the cover glass into its proper place, that is, so that it covers the ring completely. The drop of pyrogallol now spreads over the whole periphery between the cover glass and ring. Now carefully move the cover glass in the opposite direction and put a drop of potassium hydroxid upon the thus uncovered part of the ring. Replace the cover glass and make it fast. The two drops will mix and the absorption of oxygen takes place. Instead of applying the alkaline pyrogallol on the surface of the ring, it may be put on the bottom of the object carrier in the center of the ring. Later, *Nikiforoff* and *Braatz* devised special object carriers in which the hanging drop may be examined in a hydrogen atmosphere.

*Salomonson* (1889) suggests the use of aërobic bacteria as the agents for the absorption of oxygen. He uses two test tubes (Fig. 86). The smaller inner tube (a) contains the culture medium inoculated with the anaërobic species. The larger outer tube (b) with constricted neck and cotton plug holds a bouillon culture of one or more strictly aërobic species. As soon as both tubes are inoculated with their respective organisms the outer tube (b) is sealed at (c).

Besides the various apparatus described in this category, most of the apparatus for plate cultures referred to under Classes I and II may also be used for the pyrogallol method.

FIG. 86.

#### IV.

##### REDUCTION OF OXYGEN.

In order to simplify apparatus and manipulation used for cultivating anaërobic bacteria, and to be able to make cultures of anaerobes in the presence of air, attempts were made to reduce the oxygen in the medium. Various agents have been used to accomplish this end.

##### A. Mixed cultures.

One of the simplest though not altogether satisfactory ways to cultivate anaerobes under aërobic conditions is that of making mixed cultures, i. e., the culture medium containing the anaerobic species sought for is inoculated with one or more strictly aërobic species. While many investigators have used this method successfully, there seems to exist some controversy regarding the specific role which the aerobes play in preparing favorable conditions for the anaerobes in mixed cultures. *Pasteur* holds that the aërobic bacteria are capable of using up

all the oxygen present in the culture medium, and that in this way they make it possible for the anaërobes to thrive in mixed cultures. Kedrowski attributes this fact to another phenomenon. He holds that the conditions favorable for anaërobic development in mixed cultures are not due to the assimilation of the oxygen present in the nutrient medium by the aërobic species, but that the latter produce substances which form a suitable medium for the anaërobes to grow in.

*Roux* recommends the following method for mixed cultures: Boil a convenient quantity of nutrient agar in a cotton plugged test tube and cool rapidly in cold water. Immediately after the agar is solidified inoculate it with the anaërobic organism by means of a glass needle, then pour a small amount of melted but not too hot nutrient gelatin into the tube. When this is congealed pour a few drops of a bouillon culture of *Bacillus subtilis* upon the surface of the gelatin. Seal the tube in the flame and place it in the incubator. *B. subtilis* grows very rapidly upon the surface, forming a tough membrane and using up the oxygen in the tube. In order to obtain material for anaërobic subcultures, the bottom of the test tube is broken, avoiding the mixing of the two cultures.

*Penso* (1891) used a similar method. He cultivated the bacillus of malignant œdema successfully under aërobic conditions in agar and gelatin containing cultures of *Bacillus prodigiosus* or *Proteus vulgaris*.

*Scholtz* (1898) observed that anaërobes grow much more vigorously in a medium containing a vigorous culture of an aërobic species.

While it is evident that mixed cultures are a simple and generally successful means for obtaining vigorous anaërobic growth, it is equally obvious that this method is unsatisfactory where pure cultures of anaërobic bacteria must be obtained. Therefore, the use of mixed cultures can serve only as an indirect means in studying anaërobic bacteria.

#### B. Chemical reducing agents.

Glucose. *Librius*, in 1886, observed that the addition of glucose to nutrient bouillon, gelatin or agar exerted a favorable influence on the development of anaërobic species. This action has been explained by the fact that glucose in an alkaline solution possesses an oxygen-reducing power.

*Novy* (1893) successfully used and recommends media with the following ingredients for the cultivation of anaërobes:

1. Beef broth containing  $\frac{1}{2}$  per cent. sodium chloride, 2 per cent. peptone and 2 per cent. glucose.
2. Beef broth as above plus 2 per cent. gelatin.
3. 1-15 per cent. nutrient gelatin with the same constituents as under 1.
4.  $1\frac{1}{2}$  to 2 per cent. agar containing  $\frac{1}{2}$  per cent. sodium chloride, 2 per cent. peptone and 2 per cent. glucose.

*Hewlett's* method: Fill a test tube two-thirds with 2 per cent. glucose agar and steam immediately before inoculation to expel any traces of oxygen that may be present in the nutrient medium. When the agar has set, inoculate well into the depth of it. Carefully warm the tube over the flame, melting the superficial layer of agar and filling the puncture made by the inoculation. Now flame the

upper portion of the tube to expel the air and slip a well fitting rubber cap over the hot test tube.

*Babes* and *Puscarin* (1890) use high layers of two per cent. glucose agar in tubes. After inoculation they expose the tubes to 80°C. for a short time, push the plug down nearly to touch the surface of the medium and then pour liquified paraffin on top of the plug.

*Ucke* (1898) recognizes in glucose a two-edged sword, and attributes the frequent failures in the cultivation of anaërobic bacteria to the use of glucose. Although a more vigorous growth is formed on glucose medium, the spores, if formed at all, are less numerous; degeneration forms are apt to develop, and the virulence of the organism is lessened. He holds that if vigorous growth and spore producing material shall be obtained, media without sugar should be used.

Other reducing agents: *Kitasato* and *Weyl* (1890) tested the action of a large number of reducing agents in alkaline media on the development of anaërobic bacteria. Their purpose was to find a substance of a greater reducing power than glucose, and which at the same time would not exert a harmful influence on the bacterial development. They demonstrate that the addition to the culture medium of Pyrocatechin  $C_6H_4(OH)_2$ , the sodium salt of Amidonaph-tol-monosulphonic acid  $C_{10}H_6OHNH_2SO_3Na$ , sodium indigo sulphonate  $C_{16}H_8N_2O_2(SO_3Na)_2$ , Sodium formate  $HCO_2Na$ , in quantities of 0.1 per cent. exerted a favorable influence on the anaërobic growth, while other reducing reagents, such as Hydroxylaminehydrochloride  $NH_2(OH)HCl$ , Resorcin  $C_6H_4(OH)_2$ , Hydroquinone  $C_6H_4(OH)_2$ , Pyrogallol  $C_6H_3(OH)_3$ , Acetaldehyde  $CH_3COH$ , Benzaldehyde  $C_6H_5COH$ , phenylhydrazinehydrochloride  $NH_2-NH(C_6H_5HCl)$ , etc., etc., either did not affect the culture materially, or exerted a strong poisonous action on the microörganisms. The authors especially recommend the use of sodium sulphonate (use 0.1 per cent.). This agent is valuable, however, as an indicator of the reducing effect of certain bacteria rather than as a reducing agent in itself.

*Buchner* first recommended litmus to indicate changes of reaction caused by bacteria. Later, *Cohen* proposed its use as a reduction indicator. This is important, as many bacteria, and especially anaërobic forms, reduce litmus rapidly to a colorless leucosubstance, which, upon access to oxygen, colors red or blue according to the reaction changes.

*Novy* observed that the addition of litmus favors to a certain extent the growth of the microörganisms, and that it exerts a protecting influence over the anaërobic bacteria. Cultures of the bacilli of tetanus, black leg and malignant œdema retain their vitality even in liquid media exposed to the air for months if they are colored with litmus.

*Trenkmann* (1898) recommends the addition of a few drops of a ten per cent. solution of sodium sulphide  $Na_2S$  to bouillon; take 20 c. c. of nutrient medium and two drops of a ten per cent. solution of  $Na_2S$ . The author cultivated the bacillus of black leg successfully in such a medium in the presence of air. He admits, however, that  $Na_2S$  gradually decomposes by the action of  $CO_2$  in the atmosphere, forming  $Na_2CO_3$  and  $H_2S$ . As soon as this action takes place the

atmospheric oxygen reenters the medium and checks the growth of the anaërobic organisms.

*Hammerl* (1901) tested the efficiency of Trenkmann's  $\text{Na}_2\text{S}$ , of  $\text{K}_2\text{S}$  and of Ammonium sulph-hydrate  $\text{NH}_4\text{SH}$ , as reducing agents. Parallel experiments showed that  $\text{NH}_4\text{SH}$  was much more efficient than either  $\text{Na}_2\text{S}$  or  $\text{K}_2\text{S}$ . In the ammonium sulph-hydrate he believes he has found a substance which, without checking the bacterial development, reduces the oxygen in the nutrient medium and thus prepares ideal conditions for anaërobic cultures. Unfortunately the ordinary  $\text{NH}_4\text{SH}$  as kept in the laboratory is not fit for this purpose. It is necessary that it should be fresh and sterile. To obtain sterile  $\text{NH}_4\text{SH}$  the following procedure is recommended: Fill a glass stoppered bottle of 100 to 150 c. c. capacity completely with sterile water. Replace the glass stopper by a cotton plug and sterilize the apparatus in the steam sterilizer. After sterilization cool it to room temperature and introduce by means of a sterile glass tube, reaching to the bottom of the bottle, a vigorous current of washed  $\text{H}_2\text{S}$  for about six minutes. The open end of the bottle is loosely plugged with sterile cotton. Now draw from the sulphurated water accurately measured portions of 10 c. c. into each of 6-8 sterile test tubes; by means of a pipette drop into the first tube 2 drops, into the second 4 drops, etc., etc., of a 1 per cent. solution of ammonium chloride  $\text{NH}_4\text{Cl}$ . After vigorous shaking add three drops of a concentrated solution of methylene blue to each tube. The latter operation is done most easily by pouring the 10 c. c. into a tube containing the three drops of methylene blue. Mark the time required for complete decolorization. Generally the optimum amount of ammonium salt lies between 4-8 drops and the minimum time for decolorization from  $\frac{1}{4}$  to 1 minute. Upon finding the optimum amount of  $\text{NH}_4\text{Cl}$  a corresponding number of drops of a 1 per cent. solution of  $\text{NH}_4\text{Cl}$  is added to the sulphurated water in the bottle. Then add to ten parts of the nutrient medium one part of the thus prepared solution. If about three drops of concentrated methylene blue are added to this medium, the latter decolorizes rapidly and completely in 2-3 minutes.

From the above review of the various reducing agents and their comparative efficiency as a means for producing conditions favorable for the development of anaërobic bacteria in the presence of air, it may be seen that none of the agents and substances so far in use are entirely satisfactory. If aërobic species are used for this purpose, it is difficult to obtain the anaërobic in pure cultures without the aid of some other method for the cultivation of anaërobic bacteria. If we resort to chemical reducing agents we find that while they may favor anaërobic growth, they may do it at the expense of spore formation, and that they may cause the development of degeneration forms, as in the case of glucose, or the reducing substance may have a poisonous effect on the microorganisms as in the case of Hydroxylaminehydrochloride, or the preparation of the reducing agent in sterile form may be too complicated for practical purposes, as in case of Ammoniumsulph-hydrate, and finally that, no matter how great the reducing power of any one of these chemical reducing agents may be, they are not able to make harmless the atmospheric oxygen, which reenters the medium when the latter is poured into petri dishes. Generally speaking then, in order to use reducing agents successfully, they should be used in connection with some other method for cultivating anaërobic bacteria.

New York State Veterinary College, Cornell University.

OTTO F. HUNZIKER.

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The science teacher who has not provided himself with some sort of camera and has not learned how to make good reproductions of objects with it is depriving himself of one of the surest means of teaching his subject successfully. Next to the object itself, nothing is so satisfactory as an actual photograph. Descriptions and drawings are necessary, but they are more or less inaccurate and cannot give the student the clearest conception of the object. Not many schools are provided with a working museum or herbarium, but the camera in the hands of any teacher is the means of bringing a great many things to the attention and interest of students that would otherwise be unavailable. Field work with classes is more or less expensive and time consuming, and therefore cannot always be indulged in to such an extent as to be very beneficial. The habits of plants in their native haunts, differences in form of different species, differences in form of the same species under different conditions, differences at different stages of development, at different times of year, and numberless other interesting and instructive observations that cannot be brought to the class-room in any other way, may be recorded and presented almost as forcibly as though the students were taken to the field, and with far greater comfort and saving of time. The same is true of every other branch of science teaching.

With a camera one can soon accumulate a collection of illustrations from different localities that will be of no small value.

To do this does not require a great outlay. The better the tools the better the results, but even with an inexpensive box camera one may record observations that will be found exceedingly useful.

Not the least benefit derived from such elementary work is the introduction to the methods of general photography, and, what is more important, photomicrography, a subject now indispensable in every microscopical laboratory.

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The editor has had the pleasure during the past month of forwarding the applications of a number of our readers to the secretary of the American Microscopical Society, and would be glad to forward information to any others who would like to be affiliated with this, the only national society for the improvement of the microscope and the encouragement of original research in this field. The American Microscopical Society has been organized twenty-three years, and is worthy of the support of all interested in the advancement of laboratory practice.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Webber, H. J. Spermatogenesis and Fecundation of *Zamia*. U. S. Dept. of Agriculture, Bureau of Plant Industry. Bull. No. 2, pp. 1-100, pls. 1-7, 1901.

*Zamia floridana*, DC. and *Z. pumila* L., two Florida species which were heretofore incorrectly referred to *Z. integrifolia* Jacq., furnished material for this work.

Cones retain their vitality for several days—often more than a week after being removed from the plant, and so can be sent for a considerable distance and still be in perfect condition for study. Flemming's stronger solution was used for fixing archegonia, but for the pollen tube structures this solution was diluted with about four times its volume of water. It was not found necessary to bleach with peroxyde of hydrogen. The safranin-gentian violet-orange combination was the most successful stain. The spermatozooids were kept alive in a sugar solution, and their movements were carefully studied.

The most interesting portion of the paper is that which deals with the blepharoplasts. These first make their appearance in the body cell (central cell) and are formed *de novo* from the cytoplasm. They are at first very small, being scarcely more than points where a few radiating filaments converge, but as they increase in size, a surrounding membrane and vacuolated contents can be distinguished. Shortly before the division of the body cell, the nucleus passes through a synopsis stage which is regarded as normal and not at all due to reagents. The spindle is developed while the nuclear membrane is still intact, and is apparently entirely of nuclear origin and none of the fibers have any connection with the blepharoplasts. During the equatorial plate stage, the blepharoplasts break up, and in an early anaphase the contents have entirely disappeared, while the outer membrane soon breaks up into numerous granules which, during the formation of the cell plate, begin to fuse, thus forming the cilia-bearing band. At first, the band is located in the cytoplasm midway between the nucleus and the periphery of the cell, but it ultimately moves out and becomes appressed against the plasma membrane, where it forms a helicoid spiral of from five to six turns. The entire spermatid is metamorphosed into a spermatozoid, there being no differentiation of a spermatozoid within a mother cell. The mature spermatozooids are the largest known in any plant or animal, being visible to the naked eye. They move mainly by means of cilia, but there is also an amœboid movement of the spiral end.

In fertilization the entire spermatozoid enters the egg, but the nucleus soon slips out from the cytoplasmic sheath, leaving the ciliferous band in the upper part of the egg. The nucleus moves on and fuses with the egg nucleus. There is a fusion of cytoplasm with cytoplasm and nucleus with nucleus.

Prof. Webber still believes that the blepharoplast is not the homologue of the centrosphere or centrosome.

C. J. C.

Nathansohn, Alexander. Physiologische Untersuchungen über amitotische Kerntheilung. Jahrb. f. wiss. Bot. 35: 48-80, pls. 2-3, 1900.

This paper gives the results of an investigation made by the writer under Prof. Pfeffer's guidance at Leipsic.

The investigator worked almost exclusively with living material and depended chiefly upon *Spirogyra orbicularis* grown either under natural conditions or in culture dishes in the laboratory. The best results were obtained by exposing plants to a temperature of  $+2^{\circ}\text{C}$ . during the night and thus stopping cell division; the following morning growth was renewed in the warm laboratory. As soon as the first division was completed, the plants were placed in a one per cent. aqueous solution of ether, and in this continued to divide. In cells which had begun to divide indirectly, the process was completed, but amitosis followed. In order to study the behavior of the nuclei in detail, the observer fixed filaments in Flemming's solution, stained them for forty-eight hours either with dilute safranin or with Grenacher's borax-carmin, and mounted them in Venetian turpentine.

When plants which had been dividing amitotically in ether-culture were placed again under the influence of normal conditions, cells formed through amitosis divided karyokinetically and even conjugated, producing zygotes in the typical manner.

A large species of *Closterium* was grown upon agar-agar and studied both in water and in the ether solution, but the plants seldom showed amitosis. In so far as similar observations upon higher plants were concerned, the results were generally negative. Stamen hairs of *Tradescantia virginica* sometimes exhibited amitotic division of the nucleus. In the injured root tips of *Vicia Faba*, *Phaseolus multiflorus*, *Lupinus albus*, *Phalaris canadensis* and *Marsilea quadrifolia* only karyokinetic divisions were observed both in repair tissues and the vegetative point. On the contrary, in cut twigs of *Sambucus nigra* and in the injured cotyledons of *Phaseolus multiflorus* amitosis occurred. From the study of *Populus nigra*, it was judged that cells poor in cytoplasm tended to divide directly.

The author states in conclusion that the occurrence of mitosis or of amitosis does not necessarily depend upon internal peculiarities of the nuclei, but variation in this respect may be induced by changes in external conditions. He believes that cells formed through amitosis may give rise to daughter cells which possess all the embryonal qualities and undiminished power of development. Therefore he says that mitosis and amitosis may be physiologically equivalent, that karyokinesis is not necessary to a distribution of those elements which bear the hereditary qualities, and that the nucleus cannot be considered the sole possessor of those elements.

CARRIE M. DERICK.

McGill University.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Cade, A.** Etude de la constitution histologique normale et de quelques variations fonctionnelles et expérimentales des éléments sécréteurs des glandes gastriques du fond chez les mammifères. Arch. d'Anat. Microsc. 1: 1-86, 2 pls., 1901.

Studies were carried out on the dog, cat, rat, mouse, hedgehog, and marmot. Animals were in a definite physiological condition either of rest or activity, or else in a definite experi-

mental state after the use of pilocarpin or section of the vagus, etc. They were in most cases killed with chloroform, the tissues immediately removed, or else taken during chloroform narcosis. Alcohol preservation caused shrinkage. Müller's fluid, with paraffin embedding, was followed by great changes. Telejesniczki's bichromate of potash and acetic acid was useless. Aqueous 10 per cent formol, or formol in artificial serum was better. Lenhossek's mixture (sat. aqu. sublim. sol. 75 vol., absol. alcoh. 25 vol., acet. acid 5 vol.) proved better than simple aceto-sublimate. Bouin's formol-picro-acetic was most satisfactory. The stomach was taken out, opened, washed quickly in normal salt solution, and small pieces of the mucosa only, removed and put in the fixation fluid for 6 to 8 hours. Then they are passed from 60 to 90 per cent. alcohols, 24 hours in each, and embedded in paraffin, remaining in the oven half an hour. Sections are cut 10-3.3  $\mu$  in thickness and stained with hemalum and eosin, alcoholic solution, or rarely in aqueous solution. Also in hematoxylin-eosin-glycerin of Renaut; hemalum and bismark brown (aqueous 1 per cent.), iron-hematoxylin, rubin S. thionin, toluidin blue, bordeaux red, victoria blue (1 per cent. aqueous), which in much decolorized specimens is selective for granules. The different carmines, alum, borax, lithium, worked very slowly. Mucicarmine (Mayer) gave interesting preparations, used either alone or with eosin instead of hemalum. The double stain of hematein and safranin proved very important.

A. M. C.

**Kolster, R.** Paraffineinbettung im luftleeren Raume. Zeitschr. f. wiss. Mikros. u. f. mikros. Tech. 18: 70-173, 1901.

Every pathologist and histologist has to consider the possible artefacts present in tissues due to imperfections

of method. The displacement or absence of nuclei in certain cells, scratches from the knife and other changes have to be recognized. The trial of different grades of paraffin is often necessary, but with only a small amount of material this is impracticable. Concentration of the paraffin by prolonged boiling gives a good result; all water is removed and the paraffin assumes a gold-brown color, becoming harder and firmer with only a very slight change in melting point. This method gave very satisfactory results and obviates much of the trouble

coming from tearing, but still tissues occasionally show this fault. Further help is now gained by infiltrating in such a vacuum as can be obtained by the use of the pump. Any trace of the volatile liquid employed (toluol, xylol, chloroform) can be removed in such a chamber and a more solid, uniform paraffin block results. It has been possible by these means to cut the embryonic cartilaginous membranes of the spinal cord in a faultless series of sections 4–5  $\mu$  in thickness. Also the notocord of *Petromyzon* with its hard envelope, in its connection with the spinal cord, spinal ganglia, and surrounding musculature, has been cut into sections of equal or greater thinness. It was found that a different procedure was best according to the use of chloroform, xylol or toluol. With chloroform the material is passed into a warm chamber in a mixture of paraffin and chloroform, then for an equal length of time in pure paraffin, and afterwards it is put into the vacuum. If xylol or toluol is used, material is put directly from the liquid into pure paraffin and into the vacuum; only with extremely delicate tissues is it necessary to have an intermediate medium. On pumping out the air an active generation of gas results as can be seen by the bubbles formed. These gradually become smaller, and when they cease the embedding is completed.

A. M. C.

**Godlewski, J.** Ueber die Entwicklung des quergestreiften muskulösen Gewebes. Bull. de l'Acad. des Sc. de Cracovie, Classe math. et natur. 146, 1901.

Embryos of the guinea pig, rabbit, sheep, and mouse were fixed whole in Carnoy-Van Gehuchten's liquid, and

in sublimate-acetic, embedded in paraffin (52°, melting point), and cut in a continuous series from 5–10  $\mu$  in thickness, fastened to the slide with water and stained. Heidenhain's iron-hematoxylin was used with eosin or bordeaux-red for a protoplasmic stain. Many sections were treated with hemalum-eosin or vanadium-hematoxylin (after Cohn). Heidenhain's hematoxylin made visible the first appearance of the fibrillæ and their subsequent differentiation. In connection with eosin or bordeaux-red the nerves and their courses can be beautifully demonstrated.

A. M. C.

**Pranter, V.** Ein billiger Ersatz für Deckgläser. Zeitschr. f. wiss. Mikros., 18: 159–161, 1901.

Gelatin paper was suggested as a substitute for more expensive cover-glasses of large sizes. This substance is com-

posed of a pure gelatin, almost colorless, quite transparent, with a smooth surface. Paper-like sheets of 60 x 40 cm. are used in many of the arts as a protection against dust. It is soluble in water, glycerin, weak acid, and alkalies. Insoluble in strong alcohol, ether, chloroform, xylol, benzine, fatty and etherial oils. Sections in canada balsam or damar can be covered by pieces cut to the necessary size, and the oil immersion can be used without difficulty. These gelatin covers were used for temporary mounts; for permanent preparations they are less suitable, as heat and dampness causes wrinkling. This may in part be avoided if a moderately thick varnish is applied, and the surface well pressed down with blotting paper moistened in xylol. Preparations so mounted keep for a year or more perfectly well, while others not so protected spoil in a few days. For cleaning the surface soiled by the fingers a little benzine or xylol is used. These covers only in part replace glass, but prove very serviceable for large sections and temporary preparations. Grübler & Co. of Leipsic keep this substance in large pieces, and in the usual cover-glass sizes.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

- Argutinsky, P. *Malaria* studien. Arch. f. Mik. Anat. 59: 315-354, Taf. 18-21, 1901. Blood of malarial patient is spread upon the slide by Janczo and Rosenberger's method, dried at the room temperature and at once placed in sublimate-alcohol prepared as follows: Seven grains of pure sublimate are dissolved in 100 cm.<sup>3</sup> of one per cent. hot aqueous solution of common salt, and when cool 100 cm.<sup>3</sup> of absolute alcohol are added. This fixing fluid is allowed to act for 5 to 8 minutes and the slides are then washed in strong alcohol for a few minutes, and transferred to iodine alcohol (100 cm.<sup>3</sup> absolute alcohol + 2 cm.<sup>3</sup> of alcoholic tincture of iodine). This tincture is prepared by adding 1 gram of pure iodine to 100 cm.<sup>3</sup> of absolute alcohol. After rinsing in pure absolute alcohol and drying between filter paper, the slides are ready for the stain. The soda-methylen blue and eosin stain was used, prepared from methylenblau medicinale Höchst and eosin B A extra Höchst. Two methods now were employed. (1) Very dilute solutions without subsequent differentiation. Two stock solutions are made up: (a)  $\frac{1}{10}$  per cent. eosin; (b) soda-methylen blue, prepared by heating for 48 hours in a warm oven (55-60°C.) 100 cm.<sup>3</sup> of 1 per cent. methylen blue with 6 cm.<sup>3</sup> of 5 per cent. solution of soda. This can be used immediately, and remains in good condition for many days. This stain when ripened at room temperatures may be used undiluted, but is not so well adapted for staining in dilute solutions. Contrary to the recommendations of other malaria technicians, our author finds that warming the fluids during staining is unnecessary and tends to cause precipitates. Neither is it necessary to vary the proportions of the eosin and the soda-methylen blue or to use different dilutions in order to secure results with different stages of the parasite, even the most difficult. Precipitates are avoided by more dilute solution and by decreasing the proportion of eosin. The following is the author's procedure: Three cm.<sup>3</sup> of the soda-methylen blue solution prepared as above directed is mixed with 42 cm.<sup>3</sup> of distilled water. In another dish 5 cm.<sup>3</sup> of the  $\frac{1}{10}$  per cent. solution of eosin is mixed with 25 cm.<sup>3</sup> of distilled water and the two mixtures are then slowly stirred together. Slides are placed in the stains for 15 to 20 minutes. The metallic film which forms on the surface of the stain is removed with filter paper before the slides are taken from the staining dish. The preparations are washed one to two minutes in a series of dishes of distilled water, and after drying between filters are mounted in balsam.
- (2) With undiluted solutions of 1 per cent. soda-methylen blue and 1 per cent. eosin, with subsequent differentiation. The stock solutions for this method are 1 per cent. eosin and the soda-methylen blue mixture as in (1), ripened at room temperatures. After 5 days the stain may be used, and it remains in good condition for several weeks. To 15 cm.<sup>3</sup> of the 1 per cent. soda-methylen blue

solution 6 cm.<sup>3</sup> of 1 per cent. eosin are added and thoroughly mixed. This affords sufficient stain for use in a flat porcelain staining dish, in which the slide is placed preparation side down. The metallic film on the stain should be removed with filter paper before the slide is taken from the dish. It is then rinsed in water and placed in the differentiating fluid (120 cm.<sup>3</sup> 95 per cent. alcohol, to which 4 to 5 drops of glacial acetic acid and 2 cm.<sup>3</sup> of 1 per cent. aqueous solution of eosin have been added). The slide is moved to and fro with forceps and the superfluous stain comes off in blue clouds, the preparation changing from a violet to an eosin-red. Before this last trace of violet is washed out, the slide should be removed to water and thoroughly washed till no more color comes off. It is then dried between filters and mounted in balsam.

C. A. K.

**Christophers, S. R.** The Anatomy and Histology of the Adult Female Mosquito. Reports to the Malaria Committee, Royal Soc. London, Fourth Series, pp. 1-20, 6 pls., 1901.

The examination of fresh tissues reveals structures not readily discerned in fixed preparations. Dissections should be made in saline solutions of 0.3 or 0.4 per cent., as insect fluids have a lower isotonic point than those of mammals. Smear preparation of the salivary glands and mid-gut may be made from these organs after dissection. They should be spread by means of the edge of a slide or cover-glass, dried rapidly, fixed, and stained in hæmatein. Sporocysts and sporozoites, as well as other parasites in mid-gut and hind-gut, are well demonstrated by this method. Aqueous solutions should be avoided as fixing agents for whole mosquitoes on account of their failure to penetrate the exoskeleton. Absolute alcohol gave best results. As soon as the insects are partially hardened the thorax and abdomen should be opened by minute incisions to insure complete fixation of the tissues. Isolated organs are finely fixed by corrosive sublimate or picric acid. *Culex* and *Anopheles*, especially the latter cut readily in either celloidin or paraffin. Stellate cells of tracheal endings are shown by gold chloride. Hæmatein gives the best stain for the malarial parasite, and Heidenhain's hæmatoxylin is recommended for salivary glands and muscle fibres.

C. A. K.

**Stevens, N. M.** Studies on Ciliate Infusoria. Proc. Cal. Acad. Sci. Third Series; Zoölogy, 3: 1-42, pl. 1-6, 1901.

Two ciliates parasitic in the respiratory tree of *Holothuria californica* were secured in large numbers, usually together in the same host. The respiratory tree was removed from the living host, plunged into the fixing fluid, washed, hardened in alcohol, embedded and cut in sections 5 to 7  $\mu$  in thickness. For *in toto* preparations portions of the tree were stained and afterwards teased out in glycerine or clove oil. A very large number of fixing agents were tried. Hermann's fluid gave best results, though sublimate-acetic, absolute acetic, Boveri's picro-acetic, Flemming's, and Von Rath's solutions proved quite satisfactory, and osmic vapor was especially valuable for temporary *in toto* preparations for the study of division stages. Two per cent. potassium bichromate was most effective as a macerating agent, revealing internal fibre structures satisfactorily. For fresh material picro carmine and alum carmine were the best stains; for *in toto*, borax carmine, paracarmine, light green and safranin; while for sections no other stain was comparable with

Heidenhain's iron-haematoxylin following Hermann's fluid, and used either alone or in combination with rubin or ruthenium red.

C. A. K.

Needham, J. G. and Hart, C. A. The Dragonflies (*Odonata*) of Illinois; Part I, *Petaluridae*, *Aeschnidae* and *Gomphidae*. Bull. Ill. State Lab. Nat. Hist. 6: 1-94, pl. 1, 1901.

Formalin is not a good fixing agent for aquatic insects of large size, such as the nymphs of the *Odonata*. Hot alcohol

penetrates more readily, preserving internal structures from decomposition. It also serves to preserve to some extent the fugitive colors of the imago. The nymphs may be secured by examining their haunts in ponds and streams with a D-shaped dip-net. The imagos are most readily collected early in the morning. The nymphs creep up upon sticks, vegetation, piling, or boats, and the imago emerges from the larval skin. When fully dried and expanded the imagos thus secured afford the finest of specimens. Endophytic oviposition occurs among the *Zygoptera* and *Aeschnidae*, the eggs being placed by the female in the stems or leaves of aquatic vegetation. The *Gomphidae*, *Cordulegasteridae*, and *Libellulidae* deposit their eggs free in the water. Eggs of *Gomphus* are easily secured in the laboratory by holding an ovipositing female with the fore wings back to back and repeatedly touching the tip of the abdomen to a dish of water. Oviposition in captivity was not secured from other genera.

C. A. K.

Rottmann, G. Ueber die Embryonalentwicklung der Radula bei den Mollusken. I. Die Entwicklung der Radula bei den Cephalopoden. Zeitschr. f. wiss. Zool. 70: 236-262; Taf. XI, XII, 1901.

The eggs of *Loligo*, *Sepia*, *Octopus* and *Elodone* were fixed in sublimate or chrom-osmo-acetic of Flemming. To avoid the shattering of the yolk in

sectioning after long exposure to clearing fluids or the paraffin bath, the following procedure was adopted: Absolute alcohol, 24 hrs.; xylol, 2 hrs.; solution of paraffin in xylol in warm oven, ½ hr.; pure paraffin, 1 hr. Eggs thus treated were fully infiltrated and cut satisfactorily. For the demonstration of the earliest stages of the developing radula, sections previously stained in Heidenhain's iron-haematoxylin were further stained in a solution of Bismarck brown in absolute alcohol for a few minutes; the excess of stain was then washed out in absolute alcohol, and the preparations cleared in xylol, and mounted in balsam. The youngest radula is stained an intense yellowish brown, and the precision of the staining of the preparation in general enhanced by this process.

C. A. K.

Certes, A. Colorabilité élective, "intra vitam," des filaments sporifères du *Spirobacillus gigas* (Cert.) et de divers microorganismes d'eau douce et d'eau de mer par certaines couleurs d'aniline. Compt. Rend. Assoc. Franc. Av. Sci. 1901; 9 pp. 3 pl. 1901.

Sporiferous filaments of *Spirobacillus* and of many other bacteria are stained "intra vitam" by methylen blue. That of Grüber and of Höchst is most precise, but also the most toxic. *Bleu*

*d'Ehrlich* was the most readily used and gave the best results. Various Protozoa, such as *Amœba*, *Paramœcium* and other ciliates, and the suctorians *Sphaerophrya*, are also affected while living by some aniline stains, but with less certainty. The nucleus of *Amœba* is stained by acid green, or by dahlia. Malachite green stains both nucleus and the cytoplasm of *Chilodon*, *Lacrymaria*, *Spirostomum*, and *Sphaerophrya*, and the cytoplasm of *Stentor* and *Paramœcium*. In conjugation stages of the latter, however, the fragmented nucleus takes the stain. The peduncle of *Vorticella* is colored by *Bleu C 2 B de Poirrer* (1886), though not by the more recent products of this manufacturer. Diphenylamine blue, methyl blue, methylen blue and Congo red did not yield satisfactory results except in the study of food vacuoles.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Mitsukuri, K.** Negative Phototaxis and other Properties of *Littorina* as Factors in determining its Habitat. Annot. Zool. Japon. 4: 1-19, 1901.

The purpose of the author in this work was to determine the physiological factors which bring about the characteristic distribution in *Littorina exigua*

on the rocks at about the level of high tide mark. The first and most important of these factors is the marked reaction to light which the author designates as "negative phototaxis." From the account of the experiments given it would appear that the reaction is probably negative photopathy rather than phototaxis. The animals go to the region of less light intensity. This reaction would tend to cause them to move towards the land "which appeared naturally darker on account of bushes, grasses, etc." The organisms are also found to have a tendency to avoid submergence, this negative hydrotaxis working with the negative "phototaxis" to cause them to move up towards land. When experiments were so arranged as to force the animals by the light to go towards the water it was found that they would not plunge in directly, but instead would crawl horizontally along the edge. The reaction to light will eventually overcome the hydrotactic reaction. The reason for the animals being in nature uniformly distributed over the rocks is found in the habit they display of coming to rest in crevices (thigmotaxis). On glass plates they will continue moving until an obstacle is encountered. When the animal is splashed by water for some time its "phototactic" reaction in many cases becomes changed from negative to positive. This positive reaction is not as pronounced and permanent as the negative. As a result of this reaction the animals tend to move down towards the sea as the water recedes after high tide. This correlates with the fact that *Littorina* is unable to move on a perfectly dry surface, requiring a certain amount of moisture for movement and feeding. The author explains the origin of the factors in the behavior as the result of natural selection.

R. P.

**Jacobson, R.** Über die Wirkung fluoresciren-der Stoffe auf Flimmerepithel. Zeitschr. f. Biol. 41: 444-466, 1901.

This paper is a direct continuation of the work of Raab (Zeitschr. f. Biol. Bd. 39) on the action of fluorescent

substances on infusoria. The results of this investigator are now extended to ciliated epithelium. The material used was the ciliated epithelium from the mouth of the frog and the fluorescent substances tested were: (a) eosin, in concentrations of from 1:100 to 1:2000; (b) harmalin ( $C_{13}H_{14}N_2O$ ) in concentrations of from 1:1000 to 1:100000 (?); (c) akridin, in concentrations of from 1:5000 to 1:20000; (d) "Chinolinrot" ( $C_{28}H_{18}N_2Cl$ ) in solutions of 1:5000 and 1:10000. In the experiments similar pieces of living epithelium on which the cilia were actively beating were placed in dishes containing the solutions to

be tested. One set of dishes was then exposed to strong sunlight from which the heat rays had been absorbed, while the other set was kept in the dark, the temperature conditions being the same in both cases. The poisonous action of these substances (indicated by the length of time the cilia continued to beat) was found to be very markedly increased by the action of the light. The specimens kept in the dark lived in some cases five and six times as long as those exposed to the light. The action of a fluorescent, non-toxic substance asculin was not different when in light from what it was in the dark, and the same was found to be true of a toxic but non-fluorescent substance (acid fuchsin) under similar conditions. The conclusion is reached that light increases the toxic action of a fluorescent substance on ciliated epithelium.

R. P.

Greeley, A. W. On the Analogy between the Effects of Loss of Water and Lowering of Temperature. *Amer. Jour. Physiol.* 6: 122-128, 1901.

Artificial Parthenogenesis produced by a Lowering of the Temperature. *Ibid.* 6: 296-304, 1902.

In the first of these two studies on the physiological effects of low temperature it is shown that the common blue Stentor (*Stentor coeruleus*) goes into a resting condition characterized by absorption of the cilia, disappearance

of the gullet and throwing off of the ectosarc, when the temperature is gradually lowered to about 0° C. By gradually raising the temperature the organism may be brought back to its normal condition. Raising the temperature above the normal (up to 25°-28° C.) strongly stimulates cell division. If the organisms are put into an  $\frac{m}{10}$  solution of cane sugar, which of course causes a loss of water by osmosis, they shortly pass into a resting condition apparently similar to that produced by the lowering of the temperature. Similar results were obtained with Spirogyra threads. The author concludes that these experiments indicate that lowering the temperature causes the cell to lose water.

In the second paper the results of experiments on the effects of low temperature on Asterias are reported. It was found that an exposure to a temperature of from 4°-7° C. during one to nine hours will cause fully matured but unfertilized eggs of the starfish to develop into swimming larvæ. Raising the temperature failed to cause segmentation.

R. P.

Dewitz, J. Orientierung nach Himmelsrichtungen. *Arch. f. Anat. u. Physiol. Abth.* 1901. Pp. 89-105.

This paper discusses a collection of data taken principally from "Ornis" on the precise directions taken by birds

in their migration flights. It appears from the statistics given that there is a marked tendency for birds to orient exactly towards cardinal points, rather than to proceed along intermediate courses. For example, in the spring more birds fly due north than either northeast or northwest. There is apparently a positive rheotaxis of birds to the wind currents, as more fly in a given direction when the wind is coming from that direction than would under other conditions. The spatial orientations and "sense of direction" of other organisms are briefly discussed.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Leishman.** Note on a Simple Method of Producing Romanowsky Staining in Malarial and Other Blood Films. *Brit. Med. J.*, 2: Sept. 21, 1901.

**Wright.** A Rapid Method for the Differential Staining of Blood Films and Malarial Parasites. *J. of Med. Research*, 2: 138-144, 1902.

Since Jenner simplified the method for the differential staining of blood films two other workers in this field have gone further and have improved upon his work in that their stains "sharply differentiate the nuclei and the cytoplasm of the lymphocyte as well as the granulations of the leucocytes in general."

Leishman (*Brit. Med. J.*, Sept. 21, 1901) prepares his staining fluid as follows: A one per cent. methylen blue (Grübler) solution is made in distilled water, and then rendered alkaline by the addition of 0.5 per cent. of sodium carbonate. This solution is then heated to 65° C. in a paraffin oven, for twelve hours, and afterwards allowed to stand at room temperature for ten days before use. A 1 in 1000 eosin, extra B. A. (Grübler) solution is next made in distilled water. The eosin and alkaline methylene blue solutions are now mixed, in equal proportions, in a large open vessel and allowed to stand for from six to twelve hours, being stirred from time to time with a glass rod. An abundant flocculent precipitate results, which is collected on a filter and washed thoroughly with distilled water until the washings are colorless or have only a pale blue tinge. The insoluble residue is carefully collected, dried and powdered. It has a greenish metallic lustre. This powder is dissolved in the proportion of 0.15 per cent. in pure methyl alcohol (Merck's for analysis being the best) and the resulting solution is the staining fluid. It keeps well.

To stain by this method the blood films are made in the usual way and allowed to dry in the air. Three or four drops of the stain are then allowed to fall on the slide, the forceps being used to gently rotate the slide so as to ensure the stain being evenly distributed over the whole surface of the glass. No attempt should be made to check evaporation. After about a minute double the quantity of distilled water—that is, 6-8 drops—is added and allowed to mix with the stain. Intimate mixture is hastened by rotating with the forceps as above. The film is now allowed to stain for five minutes. Thick blood films or smears from cellular structures, however, may require ten minutes. The stain is next gently washed off with distilled water and a few drops of distilled water are allowed to rest on the film for one minute. This intensifies the Romanowsky staining, removes the remains of the deposit and alters the tint of the red blood corpuscles from a greenish blue to a transparent pink. The specimen is now ready for examination and may be looked at in water or mounted in Canada balsam. If distilled water be not on hand, rain or soft tap water may be employed instead. The whole operation of staining should not take more than 7-8 minutes from the time the blood is withdrawn.

By this method the *red cells* are pale pink or greenish in tint and semi-transparent; the *polynuclear leucocytes* show a nuclear network stained a ruby red color with sharply defined margins, and a colorless, extra-nuclear protoplasm with fine red granules; the *mononuclear leucocytes* have ruby red nuclei with extremely sharp, clear margins and a pale eau de-Nil or blue extra-nuclear protoplasm with occasional red granules; the *lymphocytes* are the same as the mononuclears except their nuclei are generally more deeply stained; the *eosinophilic leucocytes* exhibit a ruby red nucleus and pale pink granulations. The granules of the *basophiles* are very densely stained a deep purplish black; the nuclei are more or less masked by the granules which overlie them. The *nucleated red cells* show a nucleus almost black, with sharp outlines and a grey extra-cellular portion. The *blood plates* are of a deep ruby red with a pink margin. They frequently exhibit a pale blue peripheral zone surrounding the red center. The bodies of the *malarial parasites* are stained blue while the chromatin particles take on a ruby red color.

Wright (The Journal of Medical Research, January, 1902) has modified the above method. He thus prepares his staining fluid: A one-half per cent. watery solution of sodium carbonate is made in an Ehrlenmeyer flask and to it is added a one per cent. methylen blue (Grübler) solution. This mixture is to be steamed in an Arnold sterilizer for one hour. It is then cooled and without filtering is poured into a large disk or flask and a sufficient quantity of a 1 in 1000 solution of eosin (Grübler, yellowish, soluble in water) is added till the mixture loses its blue color and a scum with yellowish metallic lustre forms on the surface, while on close inspection a finely granular black precipitate appears in suspension. (This requires about 500 c. c. of the eosin solution for 100 c. c. of the alkaline methylen blue solution.) The precipitate is collected on a filter and allowed to dry without washing. When thoroughly dry a saturated solution is made of this powder in pure methyl alcohol. (Three-tenths of a gramme of this precipitate will thoroughly saturate 100 c. c. of methyl alcohol in a few minutes.) This saturated alcoholic solution is next filtered and twenty-five per cent. of methyl alcohol is added to the filtrate. The resulting fluid is the staining fluid, which keeps well. Care, however, should be taken that the alcohol should not evaporate.

Wright's staining method is somewhat different from Leishman's. After the blood films are made in the usual way, as much of the stain is added to the preparation as will not drain off. This is allowed to remain one minute on the slide or cover-glass before dilution. By this process the blood films are fixed. Water is then added drop by drop till the mixture becomes semi-translucent and a yellowish scum forms on the surface. This mixture should remain on the preparation for two or three minutes. By it the real staining takes place. At the end of this time the preparation is washed in water till the film assumes a yellowish or pinkish color in its better spread portions. Care should be taken lest the decolorization in water goes too far. As a rule this process takes one to two minutes. The specimen is now dried between filter paper and mounted in balsam.

The color reactions obtained by this method differ from Leishman's. Instead of a ruby red nucleus a dark lilac or blue one is seen; the granules of the polynuclear leucocytes are of a reddish lilac color. The red blood cells are orange or pink in color. For a more detailed account of the microscopical appearances of blood-films stained by this method one is referred to the original article.

W. R. S.

## NEWS AND NOTES.

Professor Morton J. Elrod, in a paper entitled, "Limnological Investigations at Flathead Lake, Montana, and Vicinity, July, 1899," describes the establishment of the biological station of the University of Montana. Flathead Lake is situated on the western side of the Mission Mountains in Montana, its northern end being about sixty miles from the British possessions. It has the general characters of Alpine lakes. It is about thirty miles long and probably not deeper than three hundred feet in the deepest place. Life is not very abundant in the lake, the record of collections made giving the names of only well known Entomostraca represented by few species. At McDonald Lake and at the ponds near the station, the same paucity of species was noticed. The expedition was rewarded by the discovery of a new land shell, *Pyramidula elrodi*, Pilsbry, and a new variety of *Limnaea emarginata*, Say, for which the varietal name *montana* has been suggested. The station was equipped with a good outfit of boats and collecting apparatus, and is to become an established department of the University of Montana. The paper is illustrated by two maps and by seven photographs of the lake and the station equipment.

In "An Addition to the Parasites of the Human Ear," Dr. Roscoe Pound describes and figures *Sterigmatocystis candida*, Sacc., which was found in the ear of a person afflicted with otitis. The paper is especially valuable, for the reason that it gives a complete list of the fungus flora of the human ear. It appears that nearly all of these parasites are Mucors or Aspergilli.

Professor Charles E. Bessey's paper on "The Modern Conception of the Structure and Classification of the Desmids" includes a revision of the tribes and a rearrangement of the North American genera. The author regards the desmids as being phylogenetically filamentous plants and, hence, related to the Zygnemaceæ, a solution of the filament leading to the development of those forms which are now found as single individuals. He includes the Zygnemaceæ, Desmidiaceæ, and Bacillariaceæ in the one order Conjugatæ. The Desmidiaceæ are then subdivided into three tribes, Desmidiæ (with cells in unbranched filaments), Arthrodieæ (with solitary, elongated cells, the latter slightly, if at all, constricted), and Cosmarieæ (with cells solitary, broad, and deeply constricted). The paper is illustrated by one plate.

"The Photo-spectrography of Colored Fluids" is the title of a short paper by Dr. Moses C. White, which draws the attention to the fact that many fluids, both colored and colorless, resemble the incandescent vapors of metals in that they, too, give characteristic lines when light passed through them is transmitted through the spectroscope. One plate accompanies the paper.

Dr. Robert H. Wolcott's "Description of a New Genus of North American Water Mites, with Observations on the Classification of the Group," is of interest to students of these organisms, not only because it contains the description of a

new species, *Steganopsis arrenuroides*, but also includes a brief critical discussion of recent attempts at a classification of the group. The new species is figured on the single plate.

"The Cladocera of Nebraska" is an important paper by Charles Fordyce, consisting of over sixty pages and four plates. The paper contains an interesting historical account of the discovery of the various species of Cladocera found in this country, the earliest date of an American publication being that of Professor S. I. Smith's "Sketch of the Invertebrate Fauna of Lake Superior, 1874," in which five species are reported; a discussion of the distribution of Cladocera throughout the state, the latter being divided into four fairly well marked faunal regions; a brief study of the vertical migration of the organisms; a synopsis of the families; and a description of about twenty-five species. The paper is a valuable contribution to the literature of this group, and will be useful to students of these organisms.

Professor Henry B. Ward continues his Notes on the Parasites of the Lake Fish in a paper entitled, "On the Structure of the Copulatory Organs of *Microphallus* nov. gen." The paper is distinctly technical in character, closing with a description of the genus, which is illustrated on the single plate.

Doctor Carl H. Eigenmann contributes a "Description of a new Cave Salamander, *Spelerpes stejnegeri*, from the Caves of Southwestern Missouri." The new species is a brightly colored form, related closely to *S. longicaudus* and *S. maculicaudus*, and is a twilight rather than a true cave species. The three species mentioned are figured on two plates.

The Limnological Commission of the Society presents an interesting report in which it makes the following suggestions: that a physicist, a chemist, and a bacteriologist be added to the Commission; that accurate systematic accounts of fresh-water organisms be published for the assistance of workers; that faunal records be kept to the end that the geographical distribution of the various species may be determined; that a summary of the work done in limnology in various countries be published from time to time; and that individual work be limited to a single body of water or to a definite problem which may concern several such bodies.

The volume closes with brief biographies and portraits of Jacob Dolson Cox and Moses Clark White, the former of whom was twice president of the Society.  
University of Rochester.

CHARLES WRIGHT DODGE.

## Books Received.

**Neurological Technique.** By Irving Hardesty, Ph. D., Instructor in Anatomy in the University of California, formerly Fellow and Assistant in Neurology in the University of Chicago. The book furnishes a collection of methods for histological investigations of the nervous system, with special attention to the details of procedure. A brief series of directions for the dissection of the mammalian brain is an important feature, together with a copy of the neurological terms adapted from the German Anatomical Society. 180 pages, 8vo, illustrated, cloth, net, \$1.75; postpaid, \$1.85.

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## THE SPERMATOZOID OF GINKGO.

### NOTES ON THE MORPHOLOGY AND METHODS.

The discovery of the spermatozoids among the Gymnosperms is one of the most remarkable events in plant morphology during the last decade of the nineteenth century. In the spring of 1896, Mr. Hirase, a Japanese botanist, found

FIG. 1-a.—Large Ginkgo tree in the Botanical Garden of the Tokyo Imperial University.

FIG. 1-b.—The Ginkgo tree, nearer view, showing the lower part of the stem.

the spermatozoids in the pollen-tube of *Ginkgo biloba*, and reported his discovery at the meeting of the Tokyo Botanical Society.<sup>1</sup> Later, in the same year, Prof. Ikeno, another Japanese botanist, discovered the spermatozoids in *Cycas revoluta*.

1. The Botanical Magazine, Tokyo, 10: 171-172, May, 1896.

The preliminary notes on these important discoveries first appeared in Japanese,<sup>1</sup> being immediately followed in German<sup>2</sup> and English.<sup>3</sup>

In the following year the occurrence of spermatozoids in *Zamia* was announced by Dr. Webber,<sup>4</sup> an American botanist. In 1900 the presence of spermatozoids in *Stangeria paradoxa*, another Cycad, was proven by an Englishman, Dr. Lang.<sup>5</sup> These discoveries may be looked upon as a partial fulfillment of the wise prophecy

of that noted German botanist Hofmeister,<sup>6</sup> who predicted more than fifty years ago the possible discovery of spermatozoids in Gymnosperms.

On the 9th of September, 1896, Mr. Hirase for the first time observed living spermatozoids of *Ginkgo* coming out from the pollen-tube. He was able to study at that time only two of them, which are figured in his full paper.<sup>7</sup> He described the spermatozoid as an oval body, 82 $\mu$  long by 49 $\mu$  wide, with a spiral marking, bearing numerous cilia on the upper end, a tail 28 $\mu$  in length on the other end, and having a large nucleus in the center completely surrounded by the cytoplasm.

The cilia on the spiral band being homologous to the tail of many animal spermatozoids, the so-called tail of the *Ginkgo* spermatozoids can not be homologized with any structure found in either

FIG. 1-c.—The same tree in winter.

animal or plant spermatozoids. Moreover, Dr. Webber has not seen any tail-like appendage in the spermatozoids of *Zamia*, and there was some hesitation among botanists in accepting the form described by Mr. Hirase as a normal one.

Our knowledge of the spermatozoids of *Ginkgo* remained in the same condition until September, 1898, when several Japanese botanists, including myself, made somewhat careful studies of them. In about a week, we examined several

1. Hirase, S.—On the spermatozoid of *Ginkgo biloba*. Bot. Mag. Tokyo, 10: 325-328, Oct., 1896.

Ikeno, S.—The spermatozoid of *Cycas revoluta*. Bot. Mag. Tokyo, 10: 367-368, Nov., 1896.

2. Hirase, S.—Untersuchungen über das Verhalten des Pollens von *Ginkgo biloba*. Bot. Centralbl. 69: 33-35, 1897.

Ikeno, S.—Vorläufige Mittheilung über die Spermatozoiden bei *Cycas revoluta*. Bot. Centralbl. 69: 1-3, 1897.

3. Ikeno and Hirase.—Spermatozoids in Gymnosperms. Ann. Bot. 11: 344, 1897.

4. Webber, H. J.—Peculiar structures occurring in the pollen-tube of *Zamia*. Bot. Gaz. 23: 453-458, 1897.

The development of the antherozoids of *Zamia*. Bot. Gaz. 24: 16-22, 1897.

5. Lang, W. H.—Studies in the development and morphology of Cycadean sporangia. II. The ovule of *Stangeria paradoxa*. Ann. Bot. 14: 281-306, 1900.

6. Hofmeister, W.—Vergleichende Untersuchungen der Keimung, Entfaltung und Fruchtbildung höherer Kryptogamen und der Samenbildung der Coniferen. Leipzig, 1851.

7. Hirase, S.—Étude sur la fécondation et l'embryogénie du *Ginkgo biloba*. Second mémoire. Jour. Coll. Sci. Imp. Univ. Tokyo, 12: 103-149, 1898.

thousands of mature ovules and were able to study over one hundred spermatozoids. The results were, that the form of spermatozoids seen, was found to be very much the same as described by Mr. Hirase, except that there is no tail. This fact was announced in a short note by Mr. Fujii<sup>1</sup> in the September number of the Botanical Magazine, and also in a note by myself<sup>2</sup> in the next number, both in Japanese. Mr. Fujii gave several figures of the spermatozoids in a later paper,<sup>3</sup> published also in Japanese.

Our observations were confirmed by Mr. Bessey, who for the first time had the opportunity of studying the living *Ginkgo* spermatozoids outside of Japan.

Since Mr. Hirase's extensive paper appeared in 1898, there was nothing written in English or any other European language about the spermatozoids of *Ginkgo*, excepting a short abstract of the paper<sup>4</sup> read by Mr. Bessey at the Baltimore meeting of the Society for Plant Morphology and Physiology in December, 1900. It is the object of the present paper to make a brief statement of the studies on *Ginkgo* spermatozoids since their discovery, and to describe briefly the methods of studying them.

*Ginkgo biloba*, the only survivor of a once flourishing group of plants, finds its native home in Japan and China, mostly in a cultivated condition. The plant is a deciduous tree with beautiful fan-shaped leaves, and may sometimes reach a height of over one hundred feet and be more than five feet in diameter. The tree is monœcious, and pollination, in middle Japan, takes place about the end of April or early in May. Fig. 1 shows a female *Ginkgo* tree in the Botanical Garden of the Tokyo Imperial University. This is the tree which furnished Mr. Hirase with the material for his famous investigations, and the material for our studies was also largely taken from this tree. The male plant which furnishes the pollen for this tree is at a distance of about half a mile, located in a Buddhist temple, outside of the garden.

The pollen from the male tree, being carried by the wind, reaches the ovule of the female, and germinates inside a small cavity, called the pollen-chamber, in the upper part of the nucellus. The mature pollen grain consists of three cells: the vegetative cell, the central cell (or antheridial cell), and the tube cell (Fig. 2 A). The first prothallial cell formed soon degenerates, and now appears merely as a cleft between the persisting second prothallial cell (Fig. 2, A<sub>v</sub>), the vegetative cell, and the outer pollen wall. After the germination of the pollen, the central cell divides into two cells,—the stalk cell and the generative cell (or body cell).<sup>5</sup> The latter enlarges and finally divides into two equal cells which form the spermatozoids (Figs. 2-5). It takes a little over four months from the pollination to the formation of spermatozoids.

1. Fujii, K.—Has the spermatozoid of *Ginkgo* a tail or not? Bot. Mag. Tokyo, 12: 287-290, 1898.

2. Miyake, K.—On the spermatozoid of *Ginkgo*. Bot. Mag. Tokyo, 12: 333-339, 1898.

3. Fujii, K.—On the morphology of the spermatozoid of *Ginkgo biloba*. Bot. Mag. 13: 260-266, 1899.

4. Bessey, E. A.—Notes on the spermatozoids of *Ginkgo*. Science, 13: 255, 1901.

Dr. Webber, in his recent monograph on "Spermatogenesis and fecundation of *Zamia*" (Bulletin No. 2, Bureau of Plant Industry, U. S. Dept. Agr. 1901) gives a somewhat careful review of Mr. Fujii's two papers.

5. Mr. Hirase describes the division of the central cell as merely a nuclear division, no wall being formed between the daughter nuclei.

The time of spermatozoid formation varies somewhat in different localities and also in different seasons. As mentioned before, in Tokyo, Mr. Hirase found the spermatozoids for the first time on September 9th, 1896. In 1897 spermatozoids were seen by Mr. Fujii on the 12th, and by Mr. Hattori on the 13th of September. In the following year spermatozoids were first observed by Mr. Shibata on September 16th; but as about half of the ovules then examined had



FIG. 2.—A. Mature pollen grain, showing the persisting vegetative cell (*v*), the central cell (*c*), the tube cell (*t*) and a remnant of the disorganized first vegetative cell (*v'*); B. Development of pollen-tube, showing the nuclei of vegetative cell (*v. n.*), stalk cell (*s. n.*), and generative cell (*g. n.*), and a part of pollen-tube; C. A later stage, the generative cell (*g. c.*) becoming larger, with minute blepharoplasts (*b*) just appeared; D. The generative cell still more enlarged, radiations having appeared around the blepharoplasts;  $\times 500$ .—After Hirase.

no indication of a pollen-tube,—the pollen-tube having been ruptured by discharging the spermatozoids—the formation of the latter must have commenced earlier than the above date. We were able to find the largest number of spermatozoids on the 17th and 18th of September, and even as late as the 22d a few were observed. In 1899 I observed several spermatozoids on September 7th.

From the above mentioned facts, we may see that in Tokyo spermatozoids of *Ginkgo* are developed between the 7th and 22d of September. This would take place later in a colder climate and earlier in warmer localities. Assuming that this would be the case, Mr. Fujii, after no more spermatozoids were to be found in Tokyo

in September, 1898, made a trip to Sendai, about two hundred miles northeast of Tokyo, to make further observations, and as he had expected, spermatozoids were still developing there, the largest number being observed on the 27th. Mr. Bessey mentions that in Washington the spermatozoids are developed between August 25th and September 10th, as extremes, the most favorable time for finding them being September 1st to 3d. It is also probable that this date may differ in different trees in the same locality; but two trees, different in size, in the botanical garden, were found to develop the spermatozoids almost simultaneously.

In the mature ovule of *Ginkgo*, unlike that of the conifers, the nucellus is reduced to a thin paper-like layer which forms a cap over the endosperm. The pollen-tube is attached to the nucellus by the finely branched, root-like tip (Fig. 5), and the whole oval or oblong tube hangs down in the space between the

nucellus and the archegonia, with the grain-end turned toward the neck of the latter (Fig. 6).

The nucleus of the generative cell divides at right angles to the axis of the pollen-tube, and after this division the whole cell is divided into two equal cells without a wall between them (Fig. 5). Two small dot-like bodies, the blepharoplasts, which appear early in the generative cell and during division, are found in both ends of the cell, a small distance from the apex of the spindle, and develop later into a spiral band with numerous cilia (Figs. 2-5). In the living generative cell,



FIG. 4.—Generative cell just dividing, the blepharoplasts being found in both poles of the cell.  $\times 260$ .—After Hirase.

these bodies are seen as refractive specks on both sides of the nucleus (Fig. 3), although no radial striations are visible, as was observed in fixed material and described by Mr. Hirase (Figs. 2D, 4, 5).

The whole contents of the generative cell is trans-

formed into two spermatozoids. After the spermatozoids are formed, the cilia on their bodies begin to vibrate and presently the whole body makes a motion, sometimes entirely changing its position, within the common wall of the mother cell (Figs. 7, 8). The presence of this cell-wall has not been mentioned by Mr. Hirase, and the fact was first proved by Mr. Fujii and myself. It is interesting to note that this wall has not been found either in *Cycas* or *Zamia*.

The correctness of our observations was questioned by Dr. Webber in his recent paper. He says: "Fujii's figure 2 seems to illustrate exactly the same appearance as that described above as occurring in *Zamia*, when the spermatozoids pull away from each other and round up so that they occupy less space, and have their original location marked by the surrounding plasma membrane of the pollen-tube. The writer believes that it may be safely concluded that *Ginkgo* corresponds with *Zamia* and *Cycas* in the metamorphosis of the entire cell, and as stated above, believes that this method of differentiation is in harmony with what is found in *Marsilia*, and probably in other ferns and lower plants." But I am unable to accept his interpretation, as a

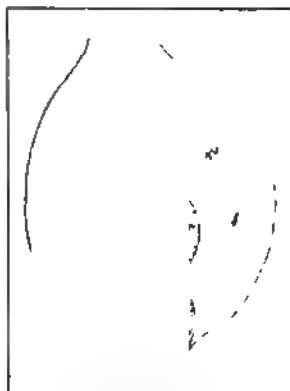


FIG. 3.—Slightly diagrammatic view of a living pollen-tube, showing the generative cell with fusiform nucleus (*n*), and the blepharoplasts (*b*); about  $\times 140$ .

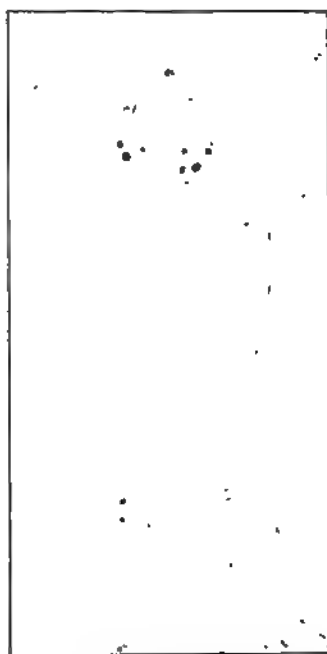


FIG. 5.—Mature pollen-tube showing its branched tip, the generative cell being already divided into two cells which form the spermatozoids;  $\times 120$ .—After Hirase.

distinct and firm wall was always found around two sister spermatozoids, as shown in Figs. 7 and 8.

In the meantime the spermatozoids come out into the wider chamber of the pollentube, breaking the wall of the mother cell. It is exceedingly fascinating to observe the spermatozoids, like a pair of large infusoria, swimming from one end of the tube to the other (Fig. 9).

The form of the spermatozoids is more or less oval, somewhat like the shape of the mature *Ginkgo* ovule, with a spiral marking, bearing cilia on the upper end. The nucleus is more hyaline and occupies a larger part of

FIG. 6.—Semi-diagrammatic section of the upper part of the endosperm, showing two archegonia (*ar.*), the beak-like process of the endosperm (*en.*) supporting the remains of the nucellus (*nc.*), and two pollen-tubes (*pt.*);  $\times 24$ .—After Hirase.

the whole body. The surrounding cytoplasmic part is more granular and has an oil-like globule of unknown nature (Figs. 9, 10). The size of spermatozoids varies somewhat in individuals. The one measured by Mr. Hirase was  $82\mu$  long by  $49\mu$  wide. According to Mr. Bessey "they are about  $105 \times 75$   $82\mu$  in size, with nucleus  $71$ – $75\mu$ ." Although I have not made very careful measure-

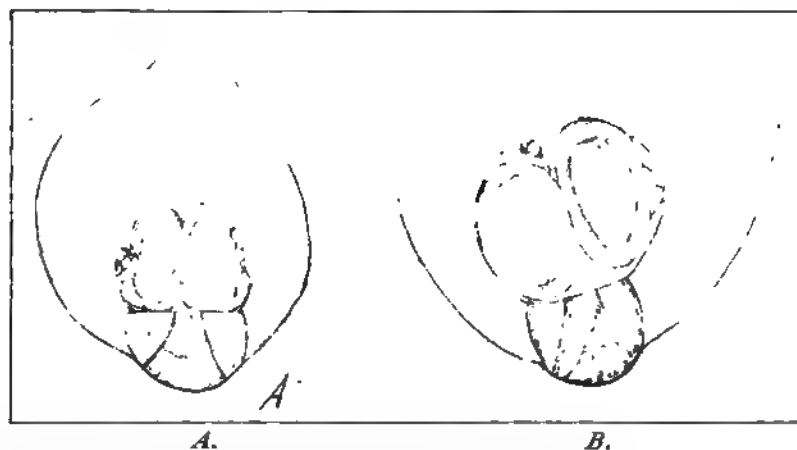


FIG. 7.—Living spermatozoids in the mother cells (optical section): *A.* Spermatozoids are gently vibrating the cilia and slightly moving their bodies; *B.* Spermatozoids are moving inside the mother cell, changing their relative positions; about  $\times 200$ .

ment, judging from several figures of spermatozoids drawn by the aid of a camera lucida, the size seems to vary from about  $110$  to  $80\mu$  in length, and from  $50$  to  $85\mu$  in width. The body of the spermatozoid is very movable, and changes form by a slight pressure, resuming the normal form as soon as the pressure is removed.

I could not find even a trace of the so-called tail-like appendage. The question naturally arises, what is the tail observed and figured by Mr. Hirase? I myself had the privilege of examining his slide of the spermatozoid with an

unmistakable tail-like appendage. Is Hirase's spermatozoid an abnormal form or an injured one? The latter alternative seems to be more probable. Mr. Fujii observed a spermatozoid form a tail-like appendage by pressure or some other cause, and also such a deformed spermatozoid resumed a normal form by absorbing the tail. I have also found a normal spermatozoid protruding a tail-like process before its death.

The spiral band on the spermatozoid seems to be of two and a half turns instead of being three, as described by Mr. Hirase and followed by Mr. Bessey (Fig. 8). In this point Mr. Fujii also agrees with me.

For examining the spermatozoids, first cut the ovule into two parts, and from the upper half take off the fleshy outer and hard inner integuments. Then a thin paper-like nucellar cap is exposed. On the somewhat pointed apex of it make a shallow cut about 3-4 mm. square with a sharp knife or razor, and peel off the part with

FIG. 8.—Two living spermatozoids in the mother cell, the spiral bands being turned toward the observer; about  $\times 200$ .

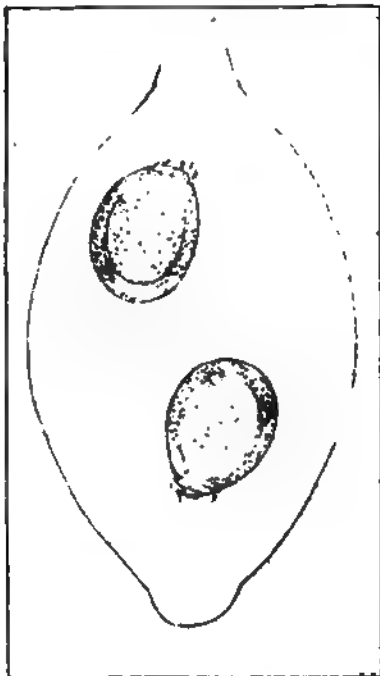


FIG. 9.—Spermatozoids swimming free in pollen-tube (somewhat diagrammatic); about  $\times 200$ .

a pair of forceps. Now in the square area of pale greenish endosperm which is thus exposed, a central process and two (sometimes three or four) small concavities will be seen. These holes are the entrance into the archegonia imbedded in the tissue of the endosperm. On the underside of the peeled piece of nucellus, one to several shining bodies may be seen. These bodies, very minute and resembling miniature dew drops, are the pollen-tubes.

Now put the square piece of the nucellus already peeled off, with the outer surface down, on a slide, and add a drop of cane sugar solution (about 6-8 per cent.)<sup>1</sup> before placing on a cover-glass. Examined under the microscope, a large cell with two spermatozoids inside may be seen at the free end of the pollen-tube (Figs. 7, 8). Sometimes instead of the spermatozoids we can see only a single oblong-elliptical or fusiform body, the nucleus, in the center of the cell (Fig. 3). This is the stage before the division of the cell to form the sperma-

1. Dr. Webber used in his studies on *Zamia* 10 per cent. solution with a good result. I found that 10 per cent. seems to be slightly strong for *Ginkgo*, 6-8 per cent. being seemingly a right concentration.

tozoids. The mother cell of the spermatozoids is attached to the grain-end of the pollen-tube by peculiar structure, called double cylinder by Mr. Hirase (Figs. 3, 7, 8). The origin and development of the double cylinder is not well understood. The similar structure found in *Zamia* has been shown, by the recent careful studies of Dr. Webber, to be double cells, the central cylinder being the vegetative cell, surrounded by the stalk cell, which forms the outer cylinder. Whether this explanation holds true in *Ginkgo* or not, is to be decided by future investigation.

FIG. 10.—Spermatozoid swimming in 10 per cent sugar solution; x 870.—After Fujii.

If we are fortunate, the first vibratory motion of the cilia, the interesting movement of spermatozoids inside the mother cell, the gradual increase of activity culminating in breaking through the mother cell, the more interesting movement of the spermatozoids swimming about inside the pollen-tube, and the final escape from the tube, can be seen in a few hours' observation. I observed two spermatozoids in active motion for two hours inside the pollen-tube, and my friend, Mr. Yabe, was able to watch one kept moving for three hours.

In *Zamia* Dr. Webber observed a spermatozoid in constant motion for two hours and forty-four minutes. The swarm-period of the spermatozoid of a fern, *Gymnogramme Martensii*, recently studied by Dr. Buller,<sup>1</sup> was found to be about two hours.

For preparing a permanent mount it would be better to imbed the spermatozoids inside the pollen-tube with a piece of nucellus attached, as it is extremely difficult to handle them after they come out of the pollen-tube. Before imbedding it should be properly fixed. For fixing I used Flemming's weak solution, Perenyi's fluid and picro-sulphuric acid solution. The first one proved to be the most satisfactory. Among several stains tried Flemming's triple stain seemed to be the best. After the specimen is soaked in the fixing fluid for about three to five hours, it is to be washed in several changes of water for five or more hours. Then stain, dehydrate, and clarify with clove-oil before finally imbedding it in canada balsam. I also found that it is sometimes better to imbed the specimen without staining. In this case spermatozoids appear brownish, due to the action of Flemming's solution, and can be well differentiated from the other part of the pollen-tube and the nucellus. The unstained preparation was found to be much better than a poorly stained specimen.

In closing I might mention a very interesting abnormal spermatozoid found by Mr. Fujii in the fall of 1899, and reported at the meeting of the Tokyo Botanical Society.<sup>2</sup> The spermatozoid has two spiral bands with a single nucleus. Its sister spermatozoid developed in the same mother cell was found to be a normal one. This abnormal form was produced probably, as Mr. Fujii suggested, by the division of a blepharoplast into two before the formation of the spiral bands. I had the good fortune of examining this very interesting form in its living condition.

K. MIYAKE.

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1. Buller, A. H. R.—Contributions to our knowledge of the physiology of the spermatozoa of ferns. *Ann. Bot.* 14: 543–582, 1900.

2. *Bot. Mag. Tokyo*, 14: 16–17, Jan., 1900.

### A Method for Cleaning Slides.

Make a strong solution of the washing powder known as "Gold Dust." Heat to the boiling point and stir down the froth that will rise to the top. This froth does no particular damage if some of it should remain, but for the sake of the esthetic sense it may be skimmed off if desired. Remove the boiling hot solution from the stove or flame, and dump in as many slides as you please to the limit of capacity of the dish. The slides may be either soft fresh ones, or old dry ones, it makes no difference to the solution. Leave them in the solution for half an hour, or until it has cooled sufficiently to work with comfortably, stirring the slides around occasionally. They will not need to be handled with the fingers. Now remove the slides to clear water and rinse. They may now be wiped dry or put through an alcohol bath and then wiped.

If the solution is hot enough and strong enough the slides will be found clean of balsam or any other mounting medium, and free from any other dirt and from all stains. If one bath should prove insufficient another one should be effective. Usually one is enough.

The slides must not be boiled, as boiling will pretty surely chip and check them, if it does not break them into bits. But the boiling hot solution will not injure them. I have never used any other washing powder, so am unable to say whether others may be as good. I use this method for practically all of the laboratory washing, especially for stained dishes, with entirely satisfactory results. I have found no stains which do not yield to the hot solution.

The advantage of this method over all others which I have tried is its cleanliness and its thoroughness in removing every vestige of the sticky balsam without the necessity of pushing the covers off or of touching the slides with the fingers before they are clean. Its death dealing qualities to all stains is enough to recommend it.

LYNDS JONES.

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### Preparation of Bone Sections.

The prerequisites for the following method of preparing bone sections are a fine-toothed saw, a triangular file, water, balsam, mucilage, slides, cover-glasses, and an oblong piece of wood, 1 x 1 x 2 cm. in size.

Cut transverse slices of tissue from the shaft of some long bone, human or otherwise, and of any convenient size, having a thickness of 2 or 3 mm. Polish one surface on the dry whetstone and stick this polished surface to a smooth side on the block of wood with mucilage. Allow it to dry thoroughly in the air or in a hot air chamber. With the saw cut away most of the section, grasp the block of wood with the fingers and rub the specimen on the wetted whetstone until it is so thin that the grain of the wood can be clearly seen through it. Now soften the mucilage by means of a little hot water if necessary to remove the specimen from the block. Place the section of bone on the wetted whetstone and rub back and forth with the ball of the finger until the papillæ thereof are distinctly visible; dry, clean by rubbing between the fingers, mount in balsam as described below, and properly label.

Place a small quantity of very thick balsam on the center of the slide, and another small quantity on the cover-glass; place the section on the slide and press the cover-glass firmly down.

Students who are unaccustomed to laboratory work make sections rapidly and well, and are able to successfully demonstrate the structure of bone. By this simple process any student not familiar with histological technique can make a longitudinal and a transverse preparation in an hour, exclusive of the time required for drying.

J. F. BURKHOLDER.

Illinois Medical College.

## LABORATORY PHOTOGRAPHY.

*Devoted to Methods and Apparatus for Converting an Object into an Illustration.*

### A FORM OF VERTICAL CAMERA AND ITS LABORATORY USES.

I have recently attempted to photograph the eggs of *Amia* at a magnification of 10 to 20 diameters. These eggs are some 2 mm. in diameter, spherical and opaque, and must be photographed under liquid with a vertical camera.

Two chief difficulties have been met with. If objectives of long focal distance be used, such as the 70 mm. of Zeiss or the 80 mm. of Leitz, the short bellows of the vertical cameras ordinarily supplied by the makers does not permit of sufficient magnification. If objectives of shorter focus be chosen, sufficient magnification is obtained, but with a loss of focal depth so that the resulting negative shows a part of the object out of focus.

The apparatus here described was devised to overcome these difficulties. It permits the use with the microscope of a vertical camera of a maximum length of 1.5 meters. The apparatus was afterward found to be of use without the microscope, i. e., in the ordinary way with photographic objectives, and is of especial value for photographing objects in liquids or objects which for any reason cannot be placed in a vertical position in front of the ordinary camera.

The camera used is the Larger Photomicrographic Camera of Zeiss (No. 235 in the English Catalogue of Instruments and Appliances for Photomicrography and Projection, 3d edition, 1898). The more recent forms of the same camera would answer as well.

This camera is made in two sections so that the front section alone may be used where a short bellows is desired. The camera is attached by means of clamps to an iron frame, consisting of two iron rods held together by cross bars at the ends and middle. By

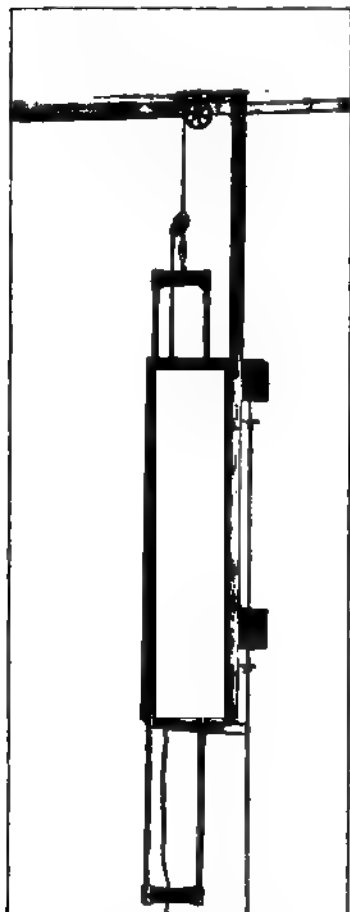


FIG. 1.—The camera has been removed from the sliding frame to show the latter in its supports. The wooden rod for focusing the camera is seen at the right. The rope seen hanging down is used in pulling down the snaffle at the end of the counterpoise rope, in order to attach it to the camera frame.

adjusting the clamps on the frame the length of the camera bellows may be varied. The frame slides back and forth in four grooved supports which are screwed to the top of an iron stand. This top is a heavy I-shaped casting bolted to the rest of the stand.

In order to use the camera in a vertical position a duplicate was made of the top of the camera stand and of the grooved supports. This was bolted to a rectangular cast iron base plate 18 x 80 cm. and 2 cm. thick. The plate had three holes along each side for  $\frac{3}{8}$ -inch lag screws. By means of these it was attached to a two-inch pine board a little larger than itself and previously screwed to the brick wall. The lower edge of the base is 90 cm. from the floor, upon which the microscope is to stand.\* (See Fig. 1.)

The camera when placed in the new vertical supports (see below) must be counterpoised. A hole is bored through the cross bar at one end of the frame and into this a brass ring is inserted. To the ring is attached a snaffle from which a heavy window cord runs through an ordinary iron pulley attached to the ceiling directly over the camera, and then through a second pulley placed in any convenient position on the ceiling. The end of the rope carries the counterpoise, equal in weight to the entire camera. If the upper section of the camera has been removed so that the lower alone may be used, then the counterpoise should be lessened by an amount equal to the upper section or else a weight equal to the weight of the upper section should be attached to the lower camera section.

In order to remove the camera from the horizontal supports the screws holding the supports of one side to the top of the camera stand should be loosened so as to permit the supports to tip outward. The side of the frame against these loosened supports should then be lifted so that the frame rotates on the opposite rod as an axis. Twisting or springing of the frame should be avoided. To place the camera in the vertical supports it should be set on end on the floor near them and the counterpoise rope snapped into place. It is convenient to have a short rope attached to the snaffle by which it may be pulled down for attachment to the camera (Fig. 1). The attached camera is then brought into position opposite the supports. The supports of one side should have been previously loosened. One side of the frame is now placed against the unloosened supports and the frame rotated on this side as an axis until the opposite side comes into place against its own supports. The screws of the loosened supports should then be tightened.

As thus arranged the camera may be used with an ordinary photographic lens attached (Fig. 2). As the sliding frame is provided with a centimeter scale, the camera may be set at the length necessary to give the desired magnification. It is convenient to construct a table of magnifications corresponding to different camera lengths. Such a table for the Zeiss Anastigmatic Objective F. 1:72,

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\* In the illustrations there is seen above the floor a platform supported on I beams, which are cemented into openings 40 cm. deep cut in the brick wall. Each I beam has holes drilled in its upper flange and through these 2 x 4 scantling are screwed to the I beams. Upon the scantling is laid a platform of 2-inch planking. The platform is thus independent of the floors and does not feel the ordinary vibration of the building. All delicate photographic work is done upon this platform.

250 mm. is given below and shows a range of possible magnifications with the lens from  $\frac{1}{10}$  to 5.

Magnification.	Camera length.
.1	26.5
.2	29.2
.3	31.5
.4	34.
.5	36.5
.6	39.
.7	41.5
.8	44.
.9	46.5
1.	48.75
2.	75.
3.	99.
4.	125.
5.	150.

Having set the camera at the desired length, the object is placed on the floor beneath and the camera may then be focused by sliding the frame up or down in the supports. A direct movement of the camera frame by the hand is apt to be jerky or to make accurate focusing difficult. To obviate this the following device is used: Alongside the camera frame (on the left) at a distance of 5 cm. from it runs a vertical wooden rod 3 to 4 cm. in diameter (Fig. 1). This rod is pivoted at its upper end to the ceiling near the first pulley wheel and at its lower end is pivoted on a wooden bracket which extends from the wall just below the board to which the iron base plate is attached. The rod is thus within easy reach of a person focusing the camera,

FIG. 2.—Showing the apparatus in use with an ordinary photographic lens, as when making Fig. 7.

and turns freely. Its upper end for about 6 cm. is formed into a spool and about it the rope running to the counterpoise makes one turn. The remainder of the rod is octagonal. By turning the rod with the hand the camera may be moved up or down with great delicacy, while the rod offers no hindrance to the direct and more rapid movement of the camera by hand. If but one section of the camera is in use care should be taken to change the counterpoise to correspond.

If the camera is conveniently situated the illumination of objects to be photographed is best accomplished by daylight or direct sunlight (see Fig. 7). We are accustomed to use the arc light. For small objects the electric arc lamp and condenser of the projection lantern with attached alum cell is made use of (as in

Fig. 8). We have obtained the best results in this and in strictly photomicrographic work by using a Thompson 90° arc lamp. With this lamp the glowing carbon is in the optical axis of the lantern so that the source of light is permanently centered. The hand feed lamp is much to be preferred to the automatic form. The lamp is supported on an adjustable stand so that it may be raised or lowered and tilted at any desired angle. The image of the glowing carbon is projected directly upon the object to be photographed.

The apparatus in this form has proved very useful for photographing such objects as cannot be readily placed in a vertical position before the horizontal camera. Preparations or dissections, fish, etc., may be photographed thus under liquid (Fig. 7).

To use the camera with the microscope it is necessary to attach the microscope to a leveling plate. This is a cast iron plate\* 17 x 25 cm. and 6 mm. thick. It is strengthened by two transverse ribs each 2.5 cm. wide and 6 mm. thick, which extend across its lower surface at a distance of 3 cm. from the ends. At its corners are truncated rectangular pyramidal feet 8 mm. high and these are pierced by leveling screws. Ear-like projections extend from either end and through them pass iron binding screws, by means of which the plate may be screwed to small iron plates set into the floor. The upper surface of the leveling plate is provided with stops against which the base of the microscope fits, with felt pads for the microscope to rest on and with a binding screw, by means of which a metal strip

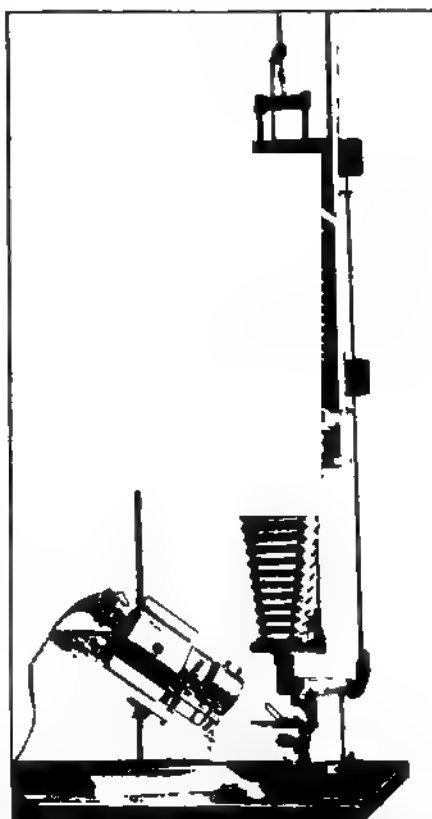


FIG. 8.—The apparatus in use with the microscope.

is clamped across the base of the microscope to hold it to the plate (Fig. 4).

To the end of the plate back of the microscope is attached a socket from which rises an iron rod 12 mm. in diameter. The rod is vertically adjustable by a thumb screw in the socket. At the top of this rod is a cross bar for bearing a pair of pulley wheels which may be set at any point on the backwardly projecting limb of the cross bar. To one end of the axis of the pulley is attached by

\* The parts of the apparatus not regularly supplied by Zeiss were made by the University mechanician, Mr. Ralph Miller, Ann Arbor, Michigan. He is prepared to furnish duplicate parts as follows: camera stand top and supports, base plates, leveling plate for microscope, focusing gear for microscope, shutter, metal pan with adjustable bottom.

means of a Hooke's key an iron rod 11 cm. long and 5 mm. in diameter (Fig. 4).

The microscope is first attached to the base plate and the latter is then placed in position beneath the camera. The camera is then lowered until the light excluding sleeve on its lower end engages with that on the microscope. The leveling screws on the plate are then adjusted until the two sleeves are concentric, as determined by feeling with the fingers that the space between the two

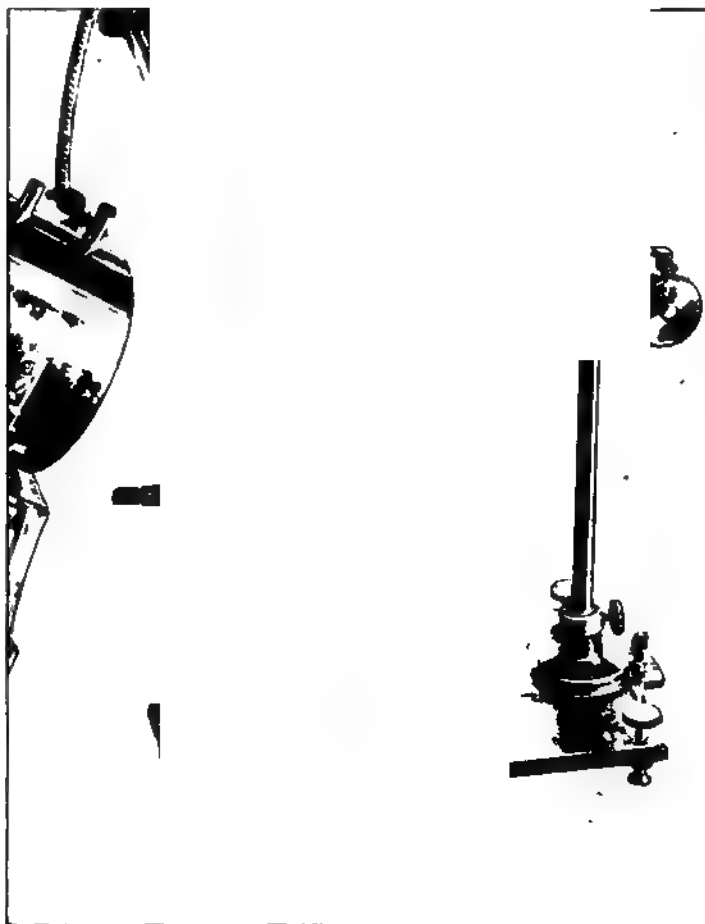


FIG. 4—Shows the details of the focusing device for the coarse adjustment of the microscope: the leveling plate; the metal pan in use; the reflector and support for focusing hair.

sleeves is of equal width on all sides. In this way the optical axis of the microscope is brought to coincide with that of the camera. The binding screws holding the leveling plate are then lightened. The pulley wheels are then connected to the coarse adjustment screws of the microscope by means of loops of linen cord, and these cords are tightened by adjusting the pulley wheels on the bar.

Supported from the wall by two brackets alongside the camera is a vertical



FIG. 4 a.—Cross section of the metal specimen pan. One-half actual size. The base of the ball and socket joint is too small, it should fill the socket.

metal rod which extends from the level of the coarse adjustment screws to the uppermost camera support (Fig. 3). The rod is provided with two milled heads which are adjustable upon it. These may be set at any point so as to be within easy reach.

At the lower end of the vertical rod is a third bracket which supports a bevel gear, the cogwheels of which are each about 3 cm. in diameter (Fig. 4). The upper wheel is attached to the vertical rod, while the lower has attached to it by means of a Hooke's key an iron rod like that attached to the axis of the pulley wheel, but only 3 cm. in length. When the leveling plate is

FIG. 5 — *Pyncnogonum Stearnsi* Ives: with egg masses. Exposure 1 minute, otherwise identical with Fig. 6.  $\times 10$ .

in position these two iron rods (that attached to the pulley wheels, and that attached to the lower part of the bevel gear) may be united by means of a brass sleeve in which are two screws (Fig. 4). The Hooke's keys give this connection a certain degree of flexibility and so permit of considerable movement of the leveling plate and of adjustment of the pulley wheels on the cross bar. When the connections have been made, the coarse adjustment of the microscope may be manipulated by an operator looking at the ground glass of the fully extended camera.

A direct connection might of course be made between the vertical rod and the coarse adjustment of the microscope, without the intervening pulleys and cords. In focusing there is some risk that the microscope tube may be brought against the lower end of the camera. If this happens the cords slip and no harm

is done. With a direct and rigid connection between the metal focusing rod and the coarse adjustment injury might result to the microscope.

This method of focusing the microscope by means of the coarse adjustment is to be preferred for all work with very low powers. Not only is it more rapid but the rapid focusing enables one to tell with much greater certainty when the object is actually in focus. With the slower movement of the fine adjustment

FIG. 6.—*Argulus Sp.* Ventral View; photographed under alcohol; B. & L. Zeiss 3-in. microplanar; stop  $\frac{1}{4}$ ; bellows length 70 cm.; lighted by arc lamp. Seed's non-halation plate, 70s. exposure. Bromo-hydrochinone developer. x 10.

one is often uncertain. If high powers are to be used, the focusing rod and Hooke's key provided with the camera may be attached to the limb of the cross bar, opposite that which carries the pulleys, and by means of these the fine adjustment may be turned from a distance.

The method of using the apparatus for photographing *small opaque objects* such as vertebrate embryos is as follows: The apparatus is set up as described

(Figs. 3 and 4), the microscope being without mirror, condenser or eyepiece. With spherical, opaque eggs it is best to attach them by means of collodion to a piece of glass. A concavity of lesser diameter than the egg is made on the glass by means of a drill. The egg is placed on this in a watch glass of ether and alcohol for a few minutes. The glass plate is then moved up against the edge of the watch glass until the egg is out of the ether and alcohol. Surplus fluid is blotted or drained off, and then by means of a slender wood or glass rod a minute drop of thin collodion is allowed to run beneath the egg by touching the glass at the point where it comes in contact with the specimen. After a moment the glass plate and egg are immersed in either alcohol or formalin. The collodion hardens almost instantly and holds the egg in place.

The glass plate is now placed on the microscope stage in a dish of alcohol or formalin and upon a background. For a light colored object, black, blue or purple glass or black velvet affords a sufficiently non-actinic background. For a darker colored object either white glass or white cardboard may be used.

For a magnification of 15 diameters the most satisfactory results have been obtained with the Bausch & Lomb-Zeiss 3-inch microplanar,\* though very good results may be had with the much cheaper 70 mm. projection lens of Zeiss or the 80 mm. projection lens of Leitz. Eyepieces are of course not used with any of the lenses.

The objective having been selected the camera length is adjusted according to the magnification desired. A table should be constructed for the purpose for each objective used.

The object is illuminated by the arc light as already described. In order to regulate the shadows a small cardboard reflector is made use of. This is attached by sealing wax to the end of a lead wire which is supported from a brass or lead disc on the microscope stage. By bending the wire the reflector may be brought into any position (Fig. 4).

Focusing is accomplished with low powers by means of the coarse adjust-

FIG. 7.—*Unio Sp.* Photographed under water. B. & L. Zeiss-Anastigmat Series II-a (No. 3). Bellows length 32.5 cm.; direct sunlight; Seed's process plate; exposure 15s. Bromo-hydrochinone developer.  $\times 1$ . Surface reflection makes this a difficult object to photograph except under water.

\* This lens as furnished by the makers has a diaphragm ring so large that it cannot be used inside the tube of the Zeiss photomicrographic stand of the older pattern. Since the lens is of too long focus to be used at the lower end of the tube of this microscope, the diaphragm ring must be filed down. The lens may then be used inside the microscope tube on the adapter furnished by Zeiss for such lenses.

ment actuated by the vertical metal focusing rod. It is best to focus first on the ground glass of the camera back and then to substitute for this the plain glass and do the final focusing by means of the focusing lens. It sometimes happens, especially with young embryos, that the object has no pronounced color differences or salient topographical features upon which to focus. Focusing is then a matter of great uncertainty and many plates are spoiled. To overcome this the following device has been used: A fine black hair from a sable brush is clamped into the split end of a lead wire supported from a base (Fig. 4) as in the case of the reflector. By bending the wire the hair is brought against the object to be photographed at about the point where it should be most sharply focused. The hair is then focused, removed, and the exposure made. This procedure saves much time.

In making the exposure a shutter is used which consists of a disc of hard rubber pivoted to a half ring of spring brass lined with chamois skin. The ring slips over the lower end of the microscope tube. This is swung to one side for the exposure by means of a little handle-like projection of the disc which extends beyond the pivot and is swung into place beneath the microscope tube at the end of the exposure (Fig. 4).

If a flat opaque object, such as a chick blastoderm, is to be photographed it is desirable to have it lie as nearly as possible in the focal plane of the objective. To accomplish this the following device has been used (Figs. 4 and 4a): To the center of a brass plate of the size and form of the microscope stage is soldered a flat brass ring 40 mm. in diameter and 9 or 10 mm. high. There is thus formed a shallow pan in the center of the brass plate. To the center of one face of a small brass disc 30 mm. in diameter is attached by a stem 2 mm. long a brass ball of about 6 mm. diameter. This ball is received into a socket between the lower face of the brass pan and a small brass disc screwed to it, and this socket is packed with oiled leather. The small metal plate then forms a sort of false adjustable bottom within the pan. This pan is filled with alcohol placed on the microscope stage, the specimen is laid on the false bottom and the bottom then tilted until the specimen lies as nearly as possible in the focal plane of the objective. The brass plate may be provided with holes, by means of which it may be attached by pins inserted into the clip-holes of the microscope stage.

The apparatus may be used with the microscope for transparent as well as for opaque objects, whenever it is necessary to photograph such objects in a horizontal position. It is merely necessary to leave the mirror and condenser of the microscope in place and throw the light from the arc lamp onto the mirror, from which it is reflected upward through the object.

In photographing vertebrate embryos as opaque objects I have found it of the greatest advantage to use *non-halation plates*. The plates render the texture and surface relief of light colored or white objects with a delicacy and truthfulness that we have been unable to approach by any other means (Figs. 5 and 6). The accompanying illustrations (Figs. 5 and 6) are made with these plates. If the object to be photographed necessitates the use of ortho-chromatic plates and a color screen, the screen may be placed in front of the illuminating apparatus or it may be placed inside the camera above the light excluding sleeve.

University of Michigan.

JACOB REIGHARD.

## AN APPARATUS FOR PHOTOGRAPHING GROSS ANATOMICAL SPECIMENS.

The following is a description of an apparatus I have found of great assistance in photographing gross anatomical specimens. Its construction is such that objects are photographed in a vertical direction, i. e., from above downward.

FIG. 1.—Photographic Apparatus. A view from the front showing camera in position.

FIG. 2.—A view from back, showing guide clamps and set screws of each carriage.

It consists of a base, a standard and two carriages, one above the other. The lower one being for the object to be photographed, and the upper for the camera.

The base is made of two by three inch stock securely jointed with machine bolts and rests on ball-bearing castors.

The standard is six feet and two inches high and consists of two uprights of three by one and one-half inch stock which stand fifteen inches apart. They are bolted to the base and firmly braced. Near the top they are joined by a cross piece. Midway between the uprights stands a heavy brass tube one and one-half inch in diameter. The lower end of this tube is secured in the base and the upper end in the cross piece.

The lower or object carriage is a light, though strong, framed bracket, the vertical part of which rests against the front surfaces of the uprights. It is fastened to the brass tube by means of two guide clamps. Each clamp is provided with milled headed set screws, which when "set up" rigidly secure the carriage at any height. On the bracket rests a wooden stage twenty-eight inches square. This has a dull ebony finish.

The upper or camera carriage (see Fig. 3) consists of three parts: a vertical frame resting against the faces of the uprights and attached to the brass tube, a bracket for the support of the camera, and a second frame for holding the camera, resting upon the upper surface of the bracket.

FIG. 3.—A view of upper or camera carriage.

Three motions are provided for in this carriage, a vertical motion and two horizontal motions at right angles to each other. The vertical motion is arranged for, as in the lower carriage, by the use of guide clamps which embrace the brass tube. In order to make the vertical adjustment of this carriage as easy as possible a counterpoise has been placed inside the brass tube and attached to the carriage by a cord passing over a pulley at the top of the tube (see Fig. 1). The bracket is attached to the vertical frame by means of what is termed by machinists a "Slide and T." The frame is ten inches broader than the bracket, thus allowing ample motion. A rack and pinion is provided for making the adjustment.

The frame for holding the camera rests on the upper surface of the bracket and is provided with a screw clamp for securing the camera firmly in position. This frame has a horizontal motion by rack and pinion also of ten inches at right angles to the one previously described. It runs on a brass slide and is held in position by a guide.

The apparatus is built of oak, oil finished, and all trimmings are of brass.

In use the object to be photographed is placed on the stage, the camera carriage moved up or down until the desired size of image is obtained, clamped and then by means of the horizontal motions of upper carriage the image is centered on the ground glass of the camera.

I have found this apparatus of particular value in photographing brains.  
Springfield, Mass.

FREDERICK S. WARD.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### V—BACILLUS OF INFLUENZA.

Influenza, popularly called "The Grip," is an important epidemic disease. The bacillus of influenza is a very small non spore forming bacillus. It occurs singly and in pairs, and occasionally several pairs are placed end to end. It is found in the secretions from the nose and in the sputum of infected persons. With the platinum loop spread a minute quantity of the nasal secretion on a clean cover-glass held in a Cornet forceps. Make a second cover preparation in the same way from the sputum. Dry the covers in the air and pass them three times, film side up, through the flame of an alcohol lamp or Bunsen burner. Stain five minutes with carbol fuchsin diluted with an equal portion of water, wash in water, dry between pieces of filter paper and mount, film side down, in a drop of balsam on a clean slip.

FIG. XIII - Bacillus influenza from nasal mucus. Stained with methyl violet. Magnified 1200 diameters with  $\frac{1}{2}$ -inch oil immersion objective. Bausch & Lomb compensating photo ocular No. 2.

### EXAMINATION OF MILK.

Milk is very susceptible to contamination at the time of milking or in the subsequent handling. It may also be filled with pathological bacteria from the animal furnishing it. To examine a sample microscopically, mix a platinum loop full of milk with an equal quantity of distilled water on a clean cover, held in a Cornet forceps. Dry over a gentle heat, holding the cover in the fingers. Stain with the following solution of methyl blue, applied on the cover:

Saturated alcoholic solution of methyl blue	-	gtts. 15
Chloroform	- - - - -	4 c. c.

Drain off surplus stain and wave the cover back and forth in the air until all of the chloroform has evaporated, then wash in water, dry and mount, film side down, in a drop of balsam on a clean slip.

When the bacillus of tuberculosis is suspected, proceed as follows: Fill the two tubes of a centrifuge with the milk and rotate the instrument at a high speed for five minutes. Decant the fluid from the sediment. Take a large platinum loop full of the sediment and mix it thoroughly on a clean cover-glass held with a Cornet forceps in twice the quantity of 1 per cent. aqueous solution of sodium carbonate. Heat carefully over a small flame till dry. This method saponifies the fat and leaves a thin film of soap over the cover. Apply a liberal quantity of carbol fuchsin, heat till it steams and after five minutes counter stain and

decolorize with Gabbet's blue, which should be allowed to act till the cover is stained blue. The bacilli of tuberculosis if present will be stained red. All other organisms will be blue.

#### URINARY ANALYSIS.

Begin and complete every examination of urine according to a general plan and file a record for reference and comparisons. The following record is one of normal urine :

Name ———— —A. B.——— Age 32 ———— Sex Male.

Address ————

Quantity voided in 24 hours	- - -	1350 c. c.—45 oz.
Color	- - -	Wine yellow
Reaction	- - -	Acid
Specific gravity	- - -	1018

#### NORMAL CONSTITUENTS.

Urea	- - -	26 grams
Uric acid	- - -	.569 grams
Chlorides	- - -	Centrifuge 11 per cent.
Phosphates	- - -	" 10 per cent.
Sulphates	- - -	" 8 per cent.

#### ABNORMAL CONSTITUENTS.

Albumin	- - -	None
Sugar	- - -	None

#### SEDIMENTS.

A few epithelial cells and debris

Casts	- - -	None
Spermatozoa	- - -	None



Urinometer.

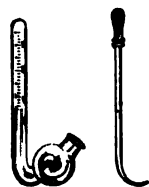
Have the patient collect all the urine he passes in twenty-four hours in a clean one-half gallon bottle, which should be kept well stoppered in a cool place. Do not trust him to mix the urine, measure it, and bring a sample, as frequent errors will result. The normal quantity of urine voided by a healthy adult, male, is 1200 to 1500 c. c., female, 1000 to 1200 c. c., children, quantities according to age. The color should be a wine yellow, but this varies widely in normal urine. The reaction should be acid. Test as follows : Immerse the ends of two strips of litmus paper, one red the other blue, in the urine. If it is neutral there will be no change of color. If acid, the blue paper will turn red. If alkaline, the red paper will turn blue. Record the reaction.

*Specific Gravity.*—Fill the jar of a urinometer about three-fourths full of urine and lower the urinometer gently in it so that it may not adhere to the side of the jar. After it has become still read the specific gravity direct from the graduated scale.

## UREA.

Urea is always present in urine of any reaction. Normally about 2 per cent. of the urine is urea. If less than 20 grams, about 300 grains, or more than 30 grams, about 450 grains, of urea are passed in 24 hours it should attract attention.

The Doremus method of determining the amount of urea is satisfactory and simple. A Doremus ureometer and the following solutions will be required :



Doremus Uriometer.

Caustic soda C. P.	-	-	100 grams.	{ 1540 grains }
Distilled water	-	-	250 c. c.	{ 8½ oz. }
Mix and allow to cool.				
Bromine	-	-	25 c. c.	(1 oz.)

Take of the caustic soda solution 10 c. c., add to it 1 c. c. of bromine, measured with the pipette accompanying the ureometer. Mix thoroughly and add 10 c. c. of distilled water. Pour the mixture, which is a freshly made solution of hypobromite, into the ureometer, inclining it so that the upright part will be filled completely. By means of the curved pipette introduce 1 c. c. of the urine into the upright portion of the instrument so that the gas as formed may not escape, but be collected at the top. The readings are made directly from the graduations, which may be in grains per ounce or in grams per litre. In this latter case the figures .01, .02, .03, etc., mean 1 per cent., 2 per cent., or 3 per cent. of each litre of urine is urea—i. e., there are 10, 20, or 30 grams of urea per litre of urine. Urea is increased in febrile diseases, acute rheumatism, acute nephritis, congestion of the kidneys, before convulsions of pregnancy, etc. It is decreased in chronic nephritis, anemia, diabetes, insipidis, etc.

Harvey Medical College.

WILLIAM H. KNAP.

## The Technique of Biological Projection and Anesthesia of Animals.

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### III. SOLAR PROJECTION APPARATUS AND ITS ADJUSTMENT.

In dealing practically with the subject of biological projection, one of the first problems to arise is, in many cases, the cost of the necessary apparatus. It is appropriate, therefore, that the first type to be described should be the least expensive. Moreover, it is possible to construct, at very little cost of time and money, a simple but useful porte-lumière which, in connection with a reasonably good microscope, will project excellent living charts and prove equally useful in chemical and physical experiments. To illustrate, having on hand a cheap plano-convex condenser four inches in diameter and a fairly good microscope with inclination joint, a projection apparatus was constructed at a cost of about two and a half dollars. The exhibitions given with the apparatus during a period of years covered quite a wide range and included successful attempts at some of the difficult problems. If purchased complete, a good porte-lumière is not very

expensive, unless an outfit of objectives is included. The objectives used on the regular laboratory microscopes are available for projection work. Solar apparatus commends itself to beginners in projection, because of the ease with which it is manipulated.

There is a little confusion in the use of the terms *porte-lumière* and *heliostat*. While both consist, essentially, of a mirror which may be rotated so as to throw a beam of light in a given direction and maintain it in a fixed position, the necessary rotary movements are performed in the *porte-lumière* by hand, but in the *heliostat* by clockwork.

The accompanying engraving shows a simple form of *porte-lumière* with a low priced projection microscope embodying suggestions by the writer. In deciding on a location for the apparatus, if there is a choice of windows, it is well to remember that the two most desirable conditions to be met are the maximum number of hours of direct sunshine per day and the longest projecting distance,

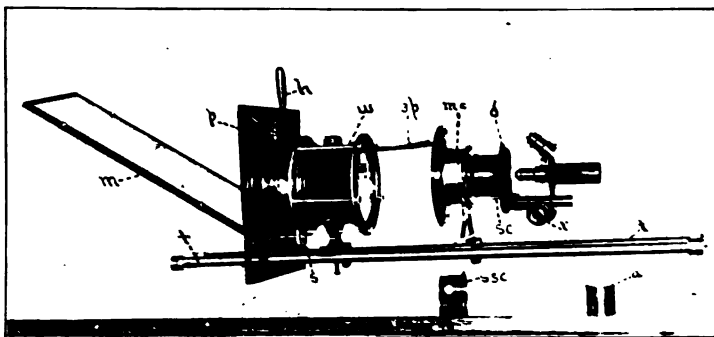


FIG. 2.—Simple Form of *Porte-lumière* and Microscope. *m*, mirror; *p*, plate; *h*, handle; *s*, mirror screw; *w*, water cell; *sp*, springs; *mc*, microscope carrier; *d*, rotary diaphragm; *sc*, spring clips; *r*, rack and pinion; *tt*, base rods; *ssc*, substage condenser; *a*, amplifier.

i. e., the distance from the *porte-lumière* to the screen. Thirty feet is a good distance and has the advantage of giving relatively high magnification when low power objectives are used, such objectives being of most general usefulness on account of their large fields and long working distances. The latter character is necessary, because, as will be explained later, live animals and plants are usually mounted in some form of glass cell containing water.

A convenient method for mounting the apparatus in the window is to cut a wide board to a length equal to the width of the window-sash. By removing a part of the batten on one side of the window, the board is set in place under the raised sash, which is then lowered until it rests upon the upper edge of the board. If the board does not fit closely so that it can be shaken, it should be fastened in place by common sliding bolts screwed to its inner side near the ends and slipping into holes carefully located in the window-frame. The plate (*p*) of the *porte-lumière* is laid on the board and marks made to indicate the position of the screw holes near the corners. A hole is then cut in the board large enough to permit the mirror (*m*) and base rods (*t*) to be passed through and the plate is

screwed fast to the board, or cleats may be fastened to the inner face of the board so as to keep the plate from slipping downward or laterally, and a strong button placed on each side will hold it firmly against the board. If the elevation of the optical axis of the porte-lumière from the floor of the room is less than the distance from the floor to the center of the picture on the screen, it is necessary to incline the plate by bringing its lower edge forward until the base rods point to a spot about six inches below the center of the screen, and then fasten it firmly, as described above.

The next step is the darkening of the room, and it is much easier and less expensive than many suppose. After successive trials of solid wooden inside shutters, black enameled cloth shades on spring rollers and the same with shallow boxes constructed around the window frame to intercept the light passing in at the edge of the shade, and finally very dark brown opaque window shade cloth on spring rollers, the latter has been found satisfactory and less troublesome than any of the others. Such an arrangement is most satisfactory on windows having a depth of four, or more inches, between the sash and the inside edge of the window frame. On shallower windows, especially if the color of the window frame is light, the edges of the shades should be boxed in. In all cases the spring roller should be set as close as possible to the sash and the shades should be as wide as the rollers can carry. These directions are not intended to produce absolute darkness in the room, for it is not necessary in order to produce brilliant pictures on the screen.

• Concerning screens it should be observed that a dead white surface is the ideal. A smoothly finished wall with a dead white paint or calcimine surface is best. Next in value as a good reflecting surface is a screen made of heavy opaque white window shade cloth. The white paint used on such a screen should be zinc white, not white lead, which soon changes to a yellow color. These screens are most conveniently mounted on spring rollers, which should always be made four inches longer than the shade is wide. Two inches of roller at each side of the shade prevents damage to the fabric if it does not always run true. The common muslin screens are troublesome on account of the difficulty of stretching them smoothly, and the large proportion of light which is lost by being transmitted through the fabric. If, however, the picture is to be observed from behind the screen, this is an advantage.

A. H. COLE.

University of Chicago.

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Permanent preparations of blood may be made by allowing fresh blood to fall drop by drop into a solution of osmic acid (two per cent. acid solution, one part; one per cent. solution of sodium chloride, two parts; distilled water, one part). The solution should be constantly stirred while the blood is dropping. Allow the blood and acid to stand one night and then wash the acid away with distilled water. Add alcohol, then clove oil, in which the blood may be kept indefinitely. Before the alcohol is added the nucleus of the corpuscle may be stained in alum-carmine, or the whole corpuscle may be stained in anilin-blue. Mount in balsam.—*Microscopical Journal*.

## LABORATORY OUTLINES.

### For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

#### VIII. *Bacteria*. Class, Schizomycetes. Order, Bacteriales.

There are three families of bacteria :

*Coccaceæ*, Spherical Bacteria, containing the genus *Micrococcus* and others.

*Bacillaceæ*, Rod Bacteria, containing the genus *Bacillus* and others.

*Spirillaceæ*, Spiral Bacteria, containing the genus *Spirillum* and others.

To obtain Bacilli, make a hay infusion by boiling ordinary dry hay for 15 minutes. Keep in a sterilized covered dish for several days. Also boil some beans, and after exposing the broth to the air until cool, cover and set aside for two or three days. Species of *Spirillum* may be obtained from sewer water, or by letting water plants decay in a jar of water. Micrococci are common in the air, and may be obtained on boiled potatoes, gelatin, moist bread, etc., by letting these culture media remain exposed for a short time and then covering them to keep in the moisture. The bacteria will soon begin to appear in yellow, pink, purple, or red patches.

1. Mount some of the hay infusion and examine under high power. Notice the minute free-swimming hay Bacillus, and draw several individuals. Draw several still hanging together in a filament after division. Describe the shape, color, and movement. Distinguish between the true locomotion of the Bacillus and the Brownian movement of the foreign particles present in the mount.

2. Draw two individuals with spores. The movement is produced by means of flagella or cilia.

3. Mount some of the bean broth and notice the putrid odor. Study the Bacillus present. Estimate the number of bacteria present in the field of the microscope. Counting the number across the diameter of the field and squaring will give approximate results.

4. Suppose you had one bacterium to begin with, and that it and its descendants divided once every hour, how many bacteria would there be at the end of each hour for 48 hours?

5. Mount some material containing *Micrococcus*. Draw several individuals and describe.

6. Mount and study some bacteria in the zoöglæa stage (bacteria in gelatinous masses). Draw and describe.

7. Mount some water containing *Spirillum*. Study the peculiar movement. Draw several individuals and describe.

8. Mount some hay Bacilli and some *Paramœcia* together, and compare them as to size. The Bacillus and the *Paramœcium* are both single cells. About how much greater in volume is the *Paramœcium* than the Bacillus? In order to get fairly accurate results, find how many times wider, thicker, and longer the one is than the other. How near would the comparison hold with that of a mouse and an elephant?

9. NOTE—To obtain Paramœcia, let a mass of Spirogyra or other water plants decay in a jar of water exposed to the air. The Paramœcium is one of the most highly developed and specialized animals belonging to the sub-kingdom Protozoa.

IX. *Slime Moulds*. Class, Myxomycetes. Order, Myxogastrales.

The Myxomycetes are a group of organisms which approach very near to the animal kingdom, forming one of the several transition groups from the lower plants to the lower animals. They have developed a very complex life history, although they are very simple plants. They usually grow on decaying logs and stumps, and may be kept dry in the encysted or resting stage for an indefinite length of time, and studied when convenient.

(a) *Lycogala epidendrum* (Buxb.) Fr.

1. Make a sketch showing the naked eye characters of individuals in the resting stage (æthalia), and how they are situated on the wood. Describe.

2. Moisten some of the downy material (capillitium) and a piece of the outer enveloping layer (peridium) in alcohol, and mount in water. Examine under high power. Is there any cell structure in the capillitium or peridium? Draw a part of the capillitium, showing the peculiar markings.

3. Draw a few of the individuals (scattered through the capillitium) in the resting stage (spores), and describe.

(b) *Hemitrichia clavata* (Pers.) Rost.

1. Mount one of the sporangia and sketch under low power, showing the stalk of the sporangium, the broken peridium, and the mass of capillitium threads. Describe shape, color, etc.

2. Under high power draw some of the capillitium threads, showing all details carefully.

3. Draw some of the individuals in the spore stage.

(c) *Stemonitis fusca* (Roth.) Rost.

1. Mount and draw one of the plume-like sporangia under the dissecting microscope, showing the hypothallus, stalk, columella, and capillitium.

2. Under high power, draw part of the capillitium, showing how it is attached to the columella.

3. Draw some of the spores.

(d) *Plasmodium*.

1. Examine under the dissecting microscope, and describe the plasmodium of a myxomycete in the moist living condition. This can usually be found on decaying logs during the spring, summer, and autumn. If living material is not at hand, examine pieces of plasmodium preserved in alcohol.

2. The flagellate stage of many species of myxomycetes may be obtained by simply making hanging drop cultures with water, or water in which decaying wood has been soaking. Fresh spores of *Lycogala* will germinate in a day or two, and the preparation can be examined from time to time under the high power.

## A Review of the Existing Methods of Cultivating Anaerobic Bacteria.

### V.

#### EXCLUSION OF ATMOSPHERIC OXYGEN BY MEANS OF VARIOUS PHYSICAL PRINCIPLES AND MECHANICAL DEVICES.

This category embraces all those methods and appliances that do not belong to any of the preceding principles. They are arranged as follows:

The atmospheric oxygen is excluded:

- A. By deep layers of solid medium.
- B. By layer of oil.
- C. By layer of paraffin.
- D. By mica or glass plates.
- E. By boiling the medium to expel the air and sealing the apparatus.
- F. By the use of the fermentation tube and its modifications.
- G. By inoculating into a hen's egg.

#### A. DEEP LAYERS OF SOLID MEDIUM.

*Hesse* (1885) and *Liborius* (1886) recommended the following method: Pour into a test tube, Erlenmeyer flask, or deep Petri dish a sufficient quantity of nutrient gelatin, nutrient agar, or blood serum to form a layer of from 5 to 20 cm. deep. Boil the medium for at least five minutes to expel the air, cool down to a temperature of about 40°C., inoculate the medium, distributing the inoculating material well through it. Care must be taken not to shake the tube or flask; after inoculation cool rapidly by standing the apparatus in cold water until the medium is set. In this way a large number of isolated colonies usually develop, which are either distributed all through the medium, or appear only in the higher zone, or inhabit exclusively the lower layers. The sharply marked distribution of the colonies over the different zones, their size and other characteristics permit a fairly accurate estimate of their relative want of oxygen. The inoculation is made with a long, firm platinum wire, which has previously been brought into contact with the culture material, or a long capillary pipette may be used, into which the culture has been introduced by suction. This simple method, which is very effective even in the case of the strictest anaërobies, is being used successfully in many laboratories.

Often a layer of sterile medium is poured on top of the inoculated layer after the latter has solidified. In order to gain access to the colonies the tube is broken. Sanfelice, 1893, warms the bottom of the tube and shakes the column of agar out into a sterile glass plate, where it can be cut into slices, the colonies can be examined and subplanted.

*Esmarch* recommends the following procedure: Make Esmarch roll cultures in the ordinary way. Stand the tubes containing the roll cultures in ice-cold water and pour liquified gelatin into the cavity of the tube. Upon cooling the

gelatin, the inoculated medium is almost entirely protected from the access of the atmosphere.

*Schill's* modification of the above method: Pour from 10 to 15 c. c. of liquified gelatin or agar into a large test tube, inoculate it with the anaërobic organism, insert a narrow sterile test tube into the inoculated medium in the large tube and roll the whole apparatus in ice water or on a cake of ice. When the operation is completed the thin layer of solidified medium in the outer tube is protected from the air by the inner, smaller test tube. Both methods have the advantage over the ordinary Esmarch roll culture that they furnish a support for the thin layer of culture medium, and prevent it from sliding down to the bottom of the tube.



FIG. 37.

### B. LAYER OF OIL.

This method, illustrated in Fig. 37, and used by Pasteur and Hesse, offers to be of little if any advantage over those described under A.

*Method.*—Pour about 7 c. c. (in case of small tube) and 15 c. c. (in case of large tube) of liquified gelatin or agar into a sterile test tube, boil to expel the air, cool to 40°C., inoculate without shaking the tube, cool rapidly in ice water until the medium is set and pour a layer of sterile oil on the surface of the inoculated medium. This method may also be used for stab cultures (see Fig. 38). Cultures prepared in this way have the disadvantage that the oil which adheres everywhere renders them somewhat objectionable.



FIG. 38.

### C. LAYER OF PARAFFIN.

*Kitasato* was the first to use paraffin as a cover over the inoculated medium. He poured liquified paraffin on the inoculated medium. This method has several disadvantages; with the paraffin foreign germs may be introduced into the medium, the neck of the culture apparatus is smeared with the paraffin, and the heat of the liquified paraffin may be injurious or even detrimental to the development of the inoculated organisms.

*Kasparec* (1896) introduced the following method: Use a flask with a long tapering neck as illustrated in Fig. 39. Blow a small lateral tube, terminating in a bulb, into the neck of the flask, about 1 cm. above (c). Fill the sterile flask with bouillon almost to the neck, then add about 3 c. c. of liquid paraffin and sterilize the whole in the steam sterilizer. The heat expands the bouillon and causes the paraffin to rise in the neck of the flask and to overflow into the lateral tube, filling the bulb (a), so that after sterilization there remains only a very thin layer of paraffin

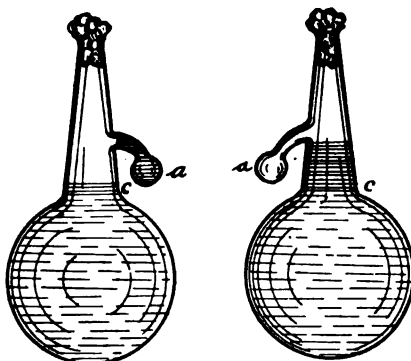


FIG. 39.

on the surface of the bouillon at (c). During the heating a large portion of the air absorbed by the medium is driven out, and its re-absorption, while the flask is cooling, is prevented by the paraffin. When cool inoculate the bouillon by piercing the solid film of paraffin with a platinum needle, loop or capillary glass tube charged with inoculating material. Now liquify the paraffin in the lateral bulb by carefully heating it over the flame and pour the liquified paraffin on the thin film of solid paraffin on the surface of the medium by inclining the flask slightly. Upon hardening this additional layer of paraffin constitutes an almost perfectly air tight cover which becomes even more effective by being pressed into the tapering neck when the culture medium is warmed in the incubator. This seal is made tighter yet by the pressure of the gases generated in the culture. When the flask is to be emptied, warm the vessel and incline the flask so that the liquified paraffin will flow over into the lateral bulb.

This method is likewise advantageous in the preparation of toxins, as the culture can be easily transferred in a very pure condition to the filter after the warmed portion of the neck of the flask has cooled and the paraffin in the lateral bulb has hardened.

*Park* (1901) modified the above method as follows: Use tubes or flasks containing sterile nutrient glucose bouillon. If non-spore-bearing anaërobes are to be cultivated, cool the medium quickly, inoculate and cover the bouillon with a layer of very hot, sterile paraffin. Where absolute exclusion of oxygen is desired sterilize the tubes containing the medium and the layer of paraffin in an autoclav; this renders the bouillon free from oxygen. Spore-bearing bacteria are inoculated through the liquid paraffin before the bouillon is cooled down. In case of bacteria without spores the medium is first cooled down and the inoculation is made by breaking through the solidified film or by heating and melting the paraffin in the gas flame. A pipette charged with the culture can then be carried through the hot paraffin into the cool liquid medium.

#### D. MICA OR GLASS PLATE.

*Koch's* method: Prepare a gelatin plate in the usual way. Before the inoculated gelatin is completely congealed cover it with a thin, sterile blade of mica. The mica blade has the thickness of a thin sheet of writing paper. It must be well flamed, and after cooling so placed upon the semi-liquid gelatin that no air bubbles form beneath it. It must cover the gelatin completely and perfectly.

*Sanfelice* modified Koch's method by using a glass plate instead of a mica plate as a cover. Pour the inoculated gelatin or agar into the sterile cover of a Petri dish and when the medium is nearly congealed press the other half of the Petri dish, bottom downward, gently into the medium.

#### E. EXCLUSION OF AIR BY BOILING THE MEDIUM AND SEALING THE APPARATUS IN THE FLAME.

*Hufner* and *Rosenbach* use a heavy 250 c. c. flask with flat bottom and tapering neck as shown in Fig. 40. Immediately below the tapering neck a lateral tube projects horizontally. At its union with the neck it measures 3 mm. in diameter. At (a) the lateral tube is constricted, then blown out into a small bulb

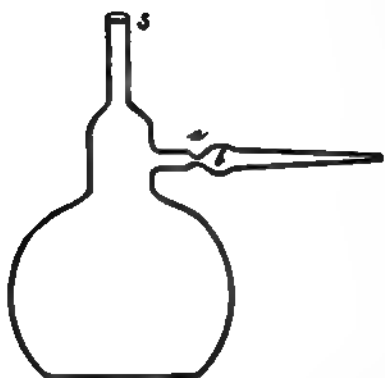


FIG. 40.

which terminates in a capillary tube 8 cm. in length. The bulb (b) constitutes the reservoir for the inoculating material.

*Method.*—Pour about 20 c. c. of medium into the flask by means of a drawnout pipette. Then taper the neck still more so that it can be sealed easily and quickly. After cooling dip the lateral tube into the inoculating material and by means of suction at (S) draw the latter up until it reaches (a). Seal the outer end of the lateral tube in the flame until a powerful stream of vapor evolves from the narrow opening of the neck. After the steam

has escaped for about four minutes seal the tapering neck in the flame. When the medium is cooled down to 40°C., slightly warm the inoculating material in the reservoir of the lateral tube. By this means it is forced into the flask, where it should be well distributed all through the medium by slightly shaking the flask. Now melt off the lateral tube at (a) and place the flask in the incubator.

*Vignal's Method.* Taper an open glass tube (Fig. 41) at one end and plug it with absorbent cotton at the other. When sterilized dip the tapered end of the sterile tube into the inoculated medium (liquified agar or gelatin) and fill the tube by suction at (a); when filled seal both ends in the flame. In order to make subcultures from the isolated colonies developed in this tube, cut the tube in two.

*Roux's pipette cultures:* (Fig. 42). The pipette consists of a glass tube drawn into a capillary tube at its lower end and constricted at (r).

*Method.*—Seal the capillary end (B) in the flame, insert a loose cotton plug at end (A) and sterilize the apparatus at 150°C. Break off the seal at (B) and dip the capillary tube into sterile, liquified nutrient agar or gelatin which has been boiled immediately before. When the tube is filled up to constriction (r), press the finger tightly over the upper opening (A) and quickly raise the tube into an oblique position. This will prevent the medium from running out. Now seal at (B) and then at (r) in the flame. For inoculation open one end, make a thrust with a fine, infected glass needle, and seal again.



FIG. 41. sterile capillary tube, then dip its open end (c) into

*Nikiforoff* (1890) constructed the capillary tube shown in Fig. 48. The apparatus consists of a reservoir (a), which is constricted and sealed at one end (b) and drawn out into a capillary tube about 25 cm. long at the other end (c). The capillary tube is U-shape. Heat the reservoir (a) of the

FIG. 42.



FIG. 43.

a test tube containing sterile water. By letting the reservoir cool, a little of the sterile water is sucked up into it. Now draw the capillary tube several times through the flame and invert it in a test tube containing the inoculated medium. The end of the capillary tube should be near but should not touch the surface of the medium. Heat the capillary tube and its reservoir, causing the enclosed water to boil, and when most of the water has escaped from it in form of vapor, immerse the open end of the capillary tube in the medium in the test tube. Upon cooling a vacuum is formed in the capillary tube and the inoculated medium is drawn up and fills the entire capillary tube and the reservoir. Now remove the test tube and seal the capillary tube at its

curve in the flame. For inoculation break the seal, introduce a fine capillary tube charged with inoculating material, and then seal the tube in the flame.

*Van Sensus* (1890) uses a glass tube about 1 m. in length and 6 mm. in diameter (Fig. 44). End (a) is drawn out into a narrow opening and wrapped up in cotton; end (b) is plugged with cotton.

*Method.*—Pour into a sterile test tube about 20 c. c. of liquified, sterile agar or gelatin and inoculate it. Remove the cotton from end (a) of the glass tube and immerse end (a) in the inoculated medium by pushing it through the cotton plug into the test tube. While the U-shape is being turned upward apply suction at end (b) till the medium reaches the curved part. Then turn the latter down, the liquid will now fill the rest of the tube by itself. Seal end (a) in the flame; the cotton plug at (b) prevents contamination.



FIG. 44.

For reaching the isolated colonies proceed as follows: Mark the place, where a well isolated colony is located, with concentrated  $H_2SO_4$  by means of a glass rod. Wash off with sterile water, scratch with file, and break the glass tube. The colony is now ready for examination and sub-plantation.

*Schmidt* (1895) uses a test tube into which a monopерforated rubber stopper is well fitted; the perforation carries a glass tube reaching to the lower surface of the rubber stopper. The glass tube extends upward about 16 cm. and is then bent into a U-shape as shown in Fig. 45.



FIG. 45.

*Method.*—Fill the sterile test tube with bouillon up to 5 mm. below the upper edge of the tube, carefully insert the rubber stopper carrying the glass tube so that the air escapes through the latter. The stopper is pushed down until the medium reaches the upper end of the glass tube. If possible prevent the flowing-over into the turned

down part of the glass tube. If gas bubbles have remained in the test tube force them out by lightly striking at the sides of the tube. If during the subsequent sterilization so much of the liquid evaporates that the glass tube has become empty, replace the evaporated medium by fresh, sterile medium. The tubes prepared in this way can be stored away indefinitely without becoming contaminated. In order to inoculate remove the stopper, inoculate the medium in the tube, and replace the stopper carefully so that the bouillon rises into the glass tube.

An ingenious and simple device has been invented by *Wright* (1901), (Figs. 46 and 47). The apparatus consists of a system of glass and rubber tubes standing in an ordinary test tube. (A), (Fig. 46), is a glass tube somewhat constricted at each end; (B), (C), and (E) are short pieces of rubber tubing; glass tube (D) carries in its upper extremity a small cotton plug. The test tube contains some culture fluid as indicated in Fig. 46.

*Method.*—Arrange the apparatus exactly as shown in Fig. 46. To expel the air from the fluid, boil the culture medium immediately before inoculation over the flame without removing the inner system of tubes. Then cool the apparatus by placing it in cold water and inoculate the liquid medium in the test tube in the usual way. Draw the fluid up into the system of glass and rubber tubes to a level above the rubber tube (C) by suction. Compress rubber tube (E) between the fingers to prevent the down-flow of the fluid, now push downward the system of tubes in such a way as to bend rubber tubes (B) and (C) in the manner shown in Fig. 47. If the test tube and the inner tube system are of suitable size the rubber tubes mentioned will remain in this bent position. The fluid in tube (A) is thus contained in a water tight space sealed by the acute angle of the rubber tubes. When it is desirable to transplant some of the culture the tube system is straightened out, this will allow the fluid in them to flow out into the test tube, where it is accessible to the platinum loop in the usual way.



FIGS. 46 and 47.

#### F. THE FERMENTATION TUBE AND ITS MODIFICATIONS.

The methods and apparatus belonging to this type deserve special mention owing to their great simplicity and efficiency.

Previous to the invention of the fermentation tube *Pasteur* devised an apparatus (Fig. 48) which operates on a similar principle. It consists of a flask (a) which contains the liquid nutrient medium. Tube (b) is conducted into a porcelain dish containing the same medium as the flask, tube (c) serves for the purpose of introducing the medium and the culture. It carries a glass turn cock (e) above which it is extended into a short rubber stoppered bulb (f) which forms the reservoir of the inoculating material.

*Method.*—Boil the medium in flask (a) and in porcelain dish (d) for one-half hour simultaneously to drive the atmospheric oxygen from the medium. The evolving vapor forces the medium out of the flask, but the liquid thus freed from air returns back into the flask. The process repeats itself several times during the boiling. When boiled for the required length of time cool apparatus and medium. While cooling cover the medium in the porcelain dish with a layer of sterile oil to prevent a subsequent absorption of oxygen

from the air. Fill (f) with the culture material and seal with the rubber stopper. When apparatus and medium are cool introduce a part of the culture material from reservoir (f) into the medium by quickly opening and closing turn cock (e). The latter must fit perfectly. Now submerge the outer end of tube (b) in sterile mercury for the purpose of collecting the gases formed by the bacterial activity, and place the apparatus in the incubator. For a control test the author uses a flask as shown in Fig. 49. It is twice as large as that shown in Fig. 48, and filled only one-half with medium so that the culture is freely exposed to atmospheric oxygen.

Smith found the fermentation tube to be an apparatus of considerable antiquity and of unknown origin. He says: "In Detmer's *pflanzenphysiologischem Practicum* I find it figured as Kühnesches Gährungsgefäß. More recently it has been adapted by Einhorn for the quantitative determination of sugar in urine and by Doremus for that of urea in the same fluid." Smith, in 1889, first conceived the value and made practical application of this tube with reference to anaërobioses and gas-formation among bacteria. The illustration (Fig. 50) represents a model fermentation tube. With regard to its construction Smith says: "In the construction of this simple bit of apparatus several points must be borne in mind. The bulb should be large enough to receive all the fluid contained in the closed branch. Moistening the plug imperils the purity of the culture. If the bulb is sufficiently large this difficulty will not arise. The connecting tube should not be too small, for then the filling and emptying of the closed branch becomes very tedious. Nor should it be too

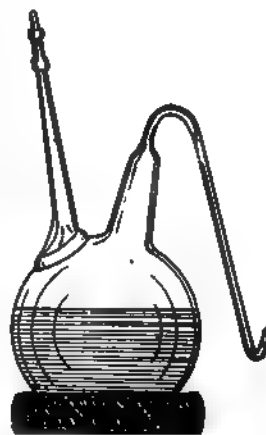


FIG. 49.

large, otherwise the anaërobic properties of the fluid in the closed branch may be less effective. Lastly, the angle formed by the two branches of the tube must not be too acute, otherwise the tube must be tilted so much during the transference of the fluid from the tube to the closed branch that there is danger of its moistening the plug or even running out of the bulb."

*Method.*—Heat the fermentation tube containing peptonized, sterile glucose bouillon in the steam sterilizer, cool, and inoculate it with the culture in question. In case of a pure anaërobic culture the growth will take place in the closed bulb and the line of demarcation between the turbid, teeming liquid of the closed branch and that of the bulb and connecting tube is sharply drawn.

In 1899 *Smith* recommended a slight modification of the above method, using the same fermentation tube.

*Method.*—Kill a guinea-pig, rabbit, pigeon or other small animal with chloroform; tear pieces of the internal organs, more particularly of the spleen, liver, and kidneys, as large as peas or beans from the organs with sterile forceps and quickly introduce them into the fermentation tubes, containing ordinary sterile, peptonized bouillon. The tissue should be eventually forced into the closed branch of the tube with a sterile platinum wire. A series of tubes are prepared at one time and placed in the incubator for several days to

FIG. 50.

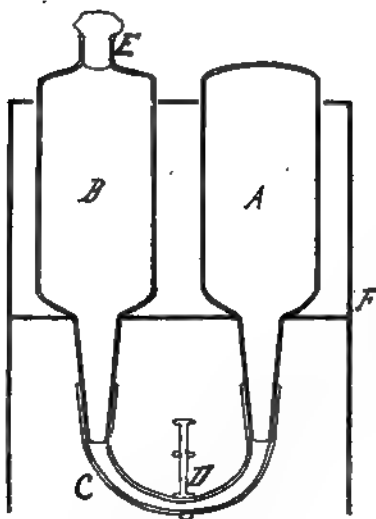


FIG. 51.

reveal any contaminating bacteria from the air or from the introduced tissue. Tubes provided in this way with bits of sterile tissues furnish most favorable conditions for the cultivation of anaërobies. They may be kept indefinitely, and when partly dried out they may be refilled with sterile water. Anaërobies will still multiply freely in them though they have not been reboiled. In fact, boiling would cloud the bouillon by coagulating the albumin from the introduced material. It is frequently very desirable to have on hand fluid cultures of anaërobic species for the study of morphological and physiological characters, a fact which makes this method especially valuable.

*Smith* constructed two more apparatus

for liquid culture media belonging to the same fermentation tube principle. Being of large capacity they are especially adapted for the cultivation, on a large scale, of the tetanus bacillus for the production of a tetanus toxin.

In apparatus illustrated in Fig. 51 there are two bulbs (A) and (B) of nearly equal capacity, connected with a heavy rubber tube (C) which carries a clamp (D) to regulate the communication between them. This apparatus is best manipulated in a tin rack (F). The bouillon occupies the whole of (A) and all below the dotted line in (B). It is inoculated by transferring the culture material with a platinum loop or pipette through the cotton plugged opening (E). The growth travels down into bulb (A) within 24 hours.

Fig. 52 represents an apparatus consisting of a stout liter flask (A), into

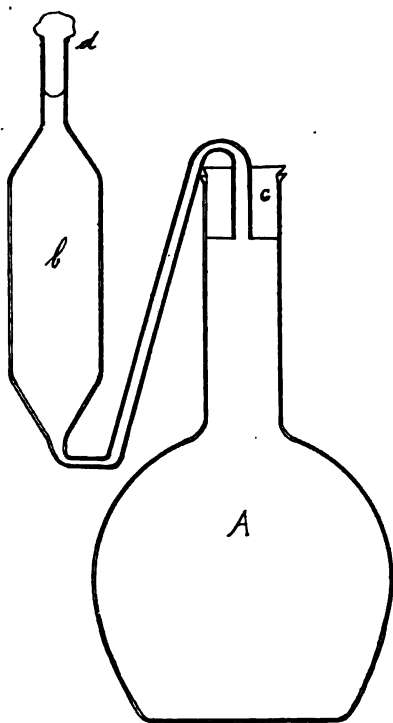


FIG. 52.

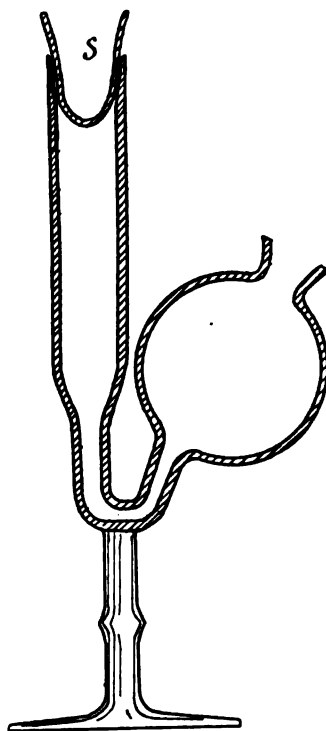


FIG. 53.

which is fitted a rubber stopper (c) carrying a 100 c. c. pipette (b) with the lower portion bent as shown in Fig. 52, and the upper shortened and provided with a cotton plug. The bouillon fills the flask completely and extends down the narrow tube to the dotted line in the bulb. The inoculation takes place through the opening (d), the growth proceeds unaided along the narrow tube and reaches the flask in from 24 to 36 hours.

The second form (Fig. 52) cannot be autoclaved when filled, as some of the fluid will be thrown out. To obviate this the flask is only partly filled and the extra bouillon required is autoclaved with it in an ordinary flask. There is no difficulty if the Arnold sterilizer is used.

*Hill* (1899) constructed the fermentation tube shown in Fig. 53. It differs from that of *Smith* in that the open bulb has twice the capacity of the closed branch. This does away with the danger of wetting the plug, when the gas pressure in the closed branch forces the liquid into the open bulb. The closed branch is sealed by means of a conical ground glass stopper (S). The stopper is made thimble-shape to avoid the danger of cracking under high temperatures, which might affect a solid stopper. This arrangement enables the experimenter to examine the liquid in the closed branch without disturbing the liquid culture. In addition to these advantages it permits a more ready and thorough cleaning, and simplifies the process of filling.

#### G. THE HEN'S EGG AS A CULTURE MEDIUM FOR ANAEROBIC BACTERIA.

*Hueppe* (1891) recommends the following procedure: Use freshly laid eggs. Clean the shell from all foreign matter; sterilize it by washing it in a solution of sublimate; rinse in sterile water and dry the shell with sterile cotton. With a flamed instrument make a small opening at the point of the egg. Through this opening inoculate by means of a platinum loop, platinum needle or capillary tube. Then cover the opening with a piece of thin, sterilized paper and seal hermetically by covering the paper with a film of collodion.

*Pearmain's and Moor's Method.*—Wash the newly laid egg in a soda solution; lay it in a  $\frac{1}{2000}$  solution of bichloride of mercury for a short time; then rinse the egg thoroughly in water that has been well boiled, finally rinse it in strong alcohol and ether immediately before inoculation. For inoculation pierce the shell with a strong sterile needle and introduce the inoculating material by means of a glass capillary tube, from which it is blown with great care, close the hole with sterile cotton wool.

*Macé* (1901) recommends shaking of the fresh egg so that the yolk mixes well with the white. Instead of just washing the egg with sublimate for a short time *Macé* lets it soak in the sublimate solution for 24 hours.

## VI.

#### COMBINED APPLICATION OF TWO OR MORE OF THE ABOVE PRINCIPLES.

Little need be said with reference to the apparatus that belong to this category. It is obvious that the large number of methods introduced permits a great variety of combinations that may be successfully used in cultivating anaerobic bacteria. Thus for instance, where it is desired to cultivate bacteria in hydrogen atmosphere, instead of forcing the air out by the current of hydrogen, the apparatus may first be partly or wholly evacuated by means of a vacuum pump, then it is connected with the Kipp generator. The exhaustion and filling may be repeated alternately several times. This combination has been used and recommended by *Pasteur*, *Novy* and other experimenters.

Where large apparatus are used, as those of *Novy* (Fig. 24), *Zubinsky* (Fig. 16), *Gabinsky* (Fig. 23), etc., a vessel containing a concentrated solution of alkaline pyrogallol may be placed in the apparatus immediately before it is sealed,

for the purpose of absorbing any traces of oxygen that may still remain in the apparatus after the current of hydrogen has been passed through. This combination is especially desirable, where it is not convenient to use a continuous current of hydrogen, in which case the apparatus is sealed when filled with the gas.

Traces of oxygen that may enter the apparatus as the result of a diffusion of the gases through imperfect seals will then be made harmless by the absorbing power of the pyrogallol.

Again, reducing agents may be added to the medium in addition to the use of any one of the other principles. The relative efficiency of the various reducing agents in use has been treated in chapter IV and need not be discussed here.

Deep layers of medium are also often used in combination with any one of the other principles.

In addition to these optional combinations *Nenki* (1880) invented the apparatus shown in Fig. 54.

*Method.*—Fill the tube up to (a) with inoculated medium and close it with a perforated rubber stopper. Through one perforation push a glass rod (b) terminating in a glass stopper of ground glass. This stopper must fit well into the constriction at (c) so that the contents of the bulb (T) are completely separated from the liquid medium above. In the second perforation insert a glass tube (d) and connect the outer end with an evacuator. During evacuation the bulb is placed in a water bath at 37°C., and the glass rod (b) is pulled up so that the stopper is located about at (a). As soon as the air is driven out, a fact which is indicated by the jerky cooking—and striking of the fluid against side of the apparatus—the glass rod is pushed down by a careful turn until the bulb is hermetically sealed.

FIG. 54.

Then, while still exhausting, the glass tube (d) is hermetically sealed in the flame. When cooled the sealed tube (d) is set in a concentrated solution of pyrogallic acid, the seal is broken, and when the pyrogallol solution reaches (n) tube (d) is again hermetically sealed.

*Vottelier's method:* Prepare about four culture tubes in the ordinary way, using solid media, when inoculated close them with loose cotton plugs which are pushed well down into the tubes. Pour 50 c. c. of an alkaline solution of pyrogallic acid into a common glass beaker. Cover the pyrogallol with a layer of liquid paraffin 2 cm. deep. Invert the culture tubes into this beaker. Introduce hydrogen into each tube for about five minutes, then place the apparatus in the incubator. In addition, this writer pours on top of the liquid paraffin a layer of the following mixture: Paraffin, solid, 50 parts; cera flava, 20 parts; vaseline, 30 parts. According to Vottelier this makes an absolutely air-tight seal. The author recommends the use of large test tubes for this purpose, as in case of small test tubes the slanted agar slides down easily. The agar must be well cooked before slanting. Before inoculation the condensation water is carefully poured off and a loose, sterile cotton plug is inserted; an occasional infection is made harmless by the pyrogallic acid.

Upon concluding this review the author's attention was drawn to a few appliances and methods not described herein. Their inventors are: *Zettnow* (1894), *Migula* (1895), *Klein* (1898), *Epstein* (1898), *Bombicci* (1902), and *Turro* (1902). These apparatus differ but slightly from those already discussed, and the methods are all based upon the principles treated in the review. Space does not permit here a detailed description of same, but the references are given in the bibliography.

### Classification of the Methods Reviewed According to the Purpose for Which They are Most Adapted.

The inventors of the existing methods and apparatus for the cultivation of anaërobic bacteria devised their apparatus and introduced their methods for certain specific purposes. While one set of apparatus may give perfect satisfaction in one line of work, it may be almost worthless elsewhere. This fact is in perfect accordance with the strong tendency for the division of labor and occupation which has so completely revolutionized the economic, industrial and scientific world of the past century. The study of anaërobic bacteria involves a series of experiments, the different stages of which vary in character. And in order to obtain rapid and satisfactory results, it is necessary that the methods employed for this work should be chosen in accordance with the purpose of their respective inventors. With the endeavor, then, of facilitating the proper selection of methods the following classification has been made:

1. Methods for the determination of the presence of anaërobic bacteria in a given substance.
2. Methods for isolating anaërobic bacteria.
3. Methods for growing pure cultures of anaërobes on different media.
4. Methods for the propagation of pure cultures and for the preservation of stock cultures.
5. Methods for the preparation of toxins.

#### 1. *Methods for the determination of the presence of anaërobic bacteria.*

The simplest and most practical methods for the study of the relation of bacteria to free oxygen consist of cultures in deep layers of solid medium as recommended by Liborius (V. a.), the use of Smith's fermentation tube (V. f.) (Fig. 50) and the use of Wright's apparatus (V. e. Figs. 46 and 47).

#### 2. *Methods for isolating anaërobic bacteria.*

To this class belong most of the methods that permit the use of solid media. In laboratories where apparatus like that of Novy (II, Fig. 24), Gabinsky (II, Fig. 23), and others are within reach, the use of plate cultures in hydrogen atmosphere is most satisfactory. In the absence of large apparatus cultures in deep layers (V. a.), Esmarch roll cultures for anaërobic bacteria as recommended by Esmarch and Schill (V. a.), Buchner's method (III, Fig. 30) are of value.

#### 3. *Methods for growing pure cultures of anaërobic bacteria on different media.*

As in the study of aërobic, so also in that of anaërobic cultures the species can be determined only by their cultural characteristics. For the determination and

differentiation of species it becomes necessary, therefore, to use various kinds of special media, and as it is of great importance, if accurate and reliable results shall be obtained, that the temperature and atmospheric conditions under which the cultures are made should be as uniform as possible, it is obvious that large apparatus, such as Gabinsky's especially, which will permit a large number of culture tubes simultaneously, are most adapted for this work.

#### 4. *Methods for the propagation of pure cultures and the preservation of stock cultures.*

Where it is desirable to have on hand cultures, fluid or solid, of anaërobic species for the study of morphological and physiological characters, it is necessary to use methods that are simple, permit prolonged growth of the organism, and from which culture material may be obtained without difficulty. As valuable methods for this purpose may be named Smith's fermentation tube containing ordinary peptonized bouillon and small sterile pieces from internal organs of rabbits, guinea-pigs, pigeons, etc. (V. f.), Wright's tube (V. e., Figs. 46 and 47), or Liborius' method (V. a.). For stock cultures of anaërobic bacteria Liborius' deep layers (V. a.) are most satisfactory. •

#### 5. *Methods for the preparation of toxins.*

Where anaërobic bacteria are cultivated for the purpose of extracting toxins from the culture medium, apparatus that permit liquid cultures in large quantities can only be considered. For this purpose Kaspavec's apparatus (V. c., Fig. 39) will serve, Park's modification of same (V. c.) and the two apparatus invented by Smith (V. f., Figs. 51 and 52).

### NUTRIENT MEDIUM.

While, generally speaking, most of the nutrient media prepared for the cultivation of aërobic bacteria will also serve, and are constantly used for anaërobic cultures, experience has shown that the latter are more exacting in their requirements of favorable media. It is not the author's intention to enter into a detailed discussion of the preparation of the media, as such instruction can be obtained from text-books and laboratory directions in bacteriology. What applies to aërobic species, will also hold true for the anaërobes. It is impossible to give a hard-and-fast rule, which embraces the media adapted for anaërobic bacteria as a group. Individual species may have their individual peculiarities which call for special media or media which have proved to be most favorable for their growth. On the other hand, it is deemed of value to draw the reader's attention to a few of the most important factors, in which the requirements of anaërobic bacteria seem to differ from those of the aërobes.

#### 1. *Composition of medium.*

Little need be said here as the composition of media has already been discussed in chapter IV on the reduction of oxygen; the same chapter contains a summary of the composition of media, which Novy (1893) found to be especially adapted for anaërobic cultures. This author, who has made a careful study of the ingredients of nutrient media that are most suited for anaërobic development,

shows that the addition of peptone greatly favors anaërobic growth. Bouillon without peptone proved to be a very poor medium, the addition of one per cent. peptone very materially improved it, and the maximum effect was reached with bouillon containing two per cent. peptone. Any addition above two per cent. did not have any noticeable effect on, or lowered the power of nutrition of such a medium. The same author also highly recommends the addition of small amounts of litmus to the nutrient medium.

Smith (1899) adds small sterile pieces of internal organs of rabbits, guinea-pigs, etc., to ordinary sterile bouillon in fermentation tubes, and successfully uses these tubes for anaërobic cultures without re-sterilizing the bouillon. In this way the medium contains some albumin in solution, and therefore offers favorable conditions for bacterial growth. (For method see V. f. Smith, 1899.)

As regards the action of sugars and other reducing agents, the reader is referred to IV.

### 2. *Reaction of Medium.*

Most of the scientists, who have studied anaërobic bacteria, agree that this group of organisms generally requires a medium with a slightly alkaline reaction. Kitasato observed that the bacillus of Rauschbrand grows best in a slightly acid medium. According to Novy, 1893, anaërobic bacteria thrive well in strongly alkaline bouillon, but in such cultures their vitality decreases rapidly and the organisms die in a comparatively short time. Thus, *Bacillus cedamatis maligni* No. 2 died within two days after inoculation, although it showed very vigorous growth at the start.

### 3. *Age of Medium.*

Since it is of great importance, if good results are to be obtained, that the medium should be as nearly free from oxygen as possible, it is obvious that old media are not suited for anaërobic cultures. The older the medium, the longer has been the time during which the latter has been absorbing oxygen from the air. It is, therefore, necessary that, for anaërobic cultures, fresh media be used. Novy (1893) Hammerl (1901) and other investigators found that in a given series of bouillon cultures the anaërobic developed much more vigorously in freshly made than in stale medium. If stale bouillon is used it should be reboiled for the purpose of driving out the oxygen present, before the medium is inoculated. The same holds true in the case of agar, while gelatin tubes evidently do not require this precaution. Thus Novy was able to cultivate anaërobic bacteria in gelatin that was six months old just as readily as in freshly made gelatin.

### 4. *Age and Quantity of the Inoculating Material.*

*a. Age.* Novy (1893), in a series of experiments with bouillon cultures of ages ranging from one day to four months, observed that where old cultures are used as inoculating material generally negative results are obtained, while, where he made the inoculations with young cultures, no difficulty was encountered in obtaining vigorous growth. He therefore concludes that the failure to secure the desired growth is often due to the age of the culture used.

*b. Quantity.* For the successful cultivation of anaërobic bacteria it is generally advisable and necessary to use large quantities of inoculating material in order to start growth. Hence, where inoculations with small amounts of culture material have proved unsuccessful, positive results may be obtained by using larger amounts of it. After the bacteria have begun to grow there is little difficulty in continuing their development.

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**SEPARATES.**

One hundred separates of each original paper accepted are furnished the author, gratis. Separates are bound in special cover with title. A greater number can be had at cost of printing the extra copies desired.

The bill introduced last December by Senator James Macmillan, authorizing the establishment of a Biological Station on the Great Lakes, under the control of the United States Commission of Fish and Fisheries, has been favorably reported, and if the members of our National legislative body are as familiar with the necessity for such a station and the improvement in the Great Lakes fisheries which would follow its establishment, as the readers of this JOURNAL, the bill will become a law, as we trust it will, without opposition. Dr. Jacob Reighard, of the University of Michigan, has been instrumental in securing the introduction of this bill and in demonstrating the necessity for such a station through his investigations on the lakes for a number of years past, and in a letter under date of April 12th, he writes in part as follows:

About \$6,600,000 is invested in the fisheries of the Great Lakes. Nearly 10,000 persons are employed, and the annual product has a value of about \$2,600,000. Commercially considered this interest is certainly great enough to warrant consideration at the hands of the government.

Concerning even the most important of the food fishes of the Great Lakes our knowledge is very meager. I may illustrate this by the following specific cases. (1) The *sturgeon* is one of the most valuable of these fishes, sturgeon products having been worth about \$81,000 in 1899; and yet we do not know enough of the spawning habits and spawning places of the sturgeon of the Great Lakes to be able to procure the eggs for artificial propagation. The sturgeon is rapidly disappearing. With adequate knowledge there is reason to believe that means might be found to increase it. (2) We know nothing of the life history of the young whitefish from the time its eggs are laid until the young whitefish are some eight inches long. A more accurate knowledge of the life history of this fish might readily lead to a rehabilitation of the fisheries, which, in some regions, are wholly depleted. In general it may be said that we know even less of the other commercial fishes than we do of the two that I have mentioned, and we know still less of the food and enemies of these fishes and of the conditions under which they pass their lives.

For the past four years the U. S. Fish Commission has endeavored to fill the gaps in our knowledge by means of studies carried on during the summer season by parties of scientific men working under the general direction of the writer. The Fish Commission has, however, no permanent establishment for carrying on this work and no funds specifically for this purpose. Although much has been accomplished by this summer work, it has served to show that the information that is of greatest practical use to the fisheries can only be obtained when work is carried on *throughout the year in a permanent establishment, with a permanent staff.*

The services of scientific men connected with the universities are available for continuous work only during a few weeks in the summer. The migrations and spawning of the commercial fishes do not take place at this time, nor are many of the more important commercial fisheries in operation at this time. It seems to me then that *a permanent scientific establishment, with an independent staff, is needed on the Great Lakes, in the interest of the fisheries.*

Such establishments the United States Fish Commission already has for the Marine Fisheries at Woods Holl, Mass., and at Beaufort, N. C. Much has been accomplished at these stations directly in the interests of the Cod, Lobster, and Oyster fisheries, besides much purely scientific work, which is certain ultimately to be of practical use to the fisheries.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Nemec, B. Die Bedeutung der fibrillären Strukturen bei den Pflanzen. Biologisches Centralblatt. 21: 529-538, 1901.

division and disappear after division is completed. The best known of these fibrillæ are those which form the achromatic figure. Another kind of fibrillæ was figured and described by Dixon in 1896, but their significance was not determined. They take no part in nuclear or cell division. Such fibrillæ are easily demonstrated in the embryo-sac mother cells of lilies.

Still another kind of fibrillæ is described by the author. Typically, these consist of an inner portion which does not stain, and a sheath which stains readily. They extend from one "Hautschicht" to the other, often around the nucleus, but sometimes entirely independent of it. In meristematic tissue they are slightly developed or even entirely lacking, but are well developed in older cells where the protoplasm is more movable. In *Allium* the fibrillæ were found to be most numerous in the region of the plasma bridges, but it must not be inferred that there is necessarily a continuity from one cell to another.

In the plerome cells of adventitious roots of *Aspidium decusatum* the fibrillæ are easily seen in the living condition. They are best examined in a two per cent. aqueous solution of grape sugar.

These fibrillæ constitute transitory, constantly changing paths which serve for the conducting of stimuli. The principal reason for assigning this function is that conduction is more rapid in the longitudinal direction, the direction of the fibrillæ, and is slow in cells where fibrillæ are absent or slightly developed.

C. J. C.

Strasburger, Ed. Das kleine botanische Practicum für Anfänger. Vierte umgearbeitete Auflage. 8vo. pp. VIII + 251 with 128 figures. Jena, Gustav Fischer, 1902. M7.

This fourth edition of the *Practicum*, like its predecessors, is remarkable for the judicious manner in which the presentation of fundamental subject matter

is flavored with modern research. As before, there are thirty-two chapters and the general method of treatment, which has proved suited to the practical needs of the laboratory, has been retained. The contents, however, have been thoroughly revised and brought up to date. The revision has been particularly thorough in the chapters on reproduction, bacteria, and nuclear and cell division. The number of types has been somewhat reduced, on the ground that it is better to study a smaller number of forms thoroughly than a larger number superficially. Some new illustrations have been added.

In this connection it may not be out of place to call attention to a book which the ordinary reader might mistake for an English translation of Prof. Strasbur-

ger's *Practicum*.\* In the first edition of the translation the translator added some notes of his own and also some additional figures, but all such matter was clearly indicated by brackets. In the second edition many brackets were removed, and in the last edition all brackets are dispensed with and the translator's notes have been incorporated into the text, so that in reading the book no one can distinguish what rests upon Strasburger's authority from that which has been inserted by the translator. This is particularly unfortunate, since in all the German editions stress has been laid upon the fact that Prof. Strasburger has drawn all the figures, and that all statements, even when they concern matters of common knowledge, rest upon his own investigations.

While the fact that the English book has reached a fifth edition indicates that there is a demand for Strasburger's *Practicum* in English, we think it would have been far better to have presented a faithful translation with an appendix suggesting English and American types which could be used where the German forms are not available.

C. J. C.

Wettstein, I. R. von. Der gegenwärtige Stand unserer Kenntnisse betreffend die Neubildung von Formen im Pflanzenreiche. Ber. d. deutsch. bot. Gesell. 18: 184-200, 1901.

A study of the literature of the origin of forms shows that during the past century logical deduction and theorizing have often been more prominent

than direct experiment and observation. Such a study also shows that botanists have contributed comparatively little to the subject.

Characters of great constancy, which are affected but little or not at all by life conditions, the writer designates as "organic characters" (Nägeli's Organisationsmerkmale). Another set of characters are designated by the term "adaptive characters." Most family, genus, and many species characters belong to the first category, but characters of races and individuals are also included; many species characters and most race and individual characters belong to the second category.

Both categories of characters are always to be distinguished in plants, but it is not always easy to decide to which category a given character belongs. Some plants, like *Pteris aquilina*, have but few adaptive characters and consequently appear about the same in all conditions, while others, like *Potentilla*, respond so readily to external factors that a great variety of forms is produced.

The author's summary is, in part, as follows: The degree of organization of a plant is to be referred to inner causes. Organic characters can be changed by the fixing of adaptive characters, by crossing and especially by *heterogenesis* (the sudden appearance of new forms). In the production of adaptive characters external factors are predominant, while crossing and heterogenesis—especially the former—play a subordinate role. This holds for natural conditions; under domestication, artificial selection exerts an important influence in the production of new forms, but in nature, selection can have but little and at most an indirect significance, being efficient, as a rule, only in so far as it removes the unfit.

C. J. C.

\* Handbook of Practical Botany, for the laboratory and the private student, by Prof. E. Strasburger. Translated and edited from the German with many additional notes by W. Hillhouse. 5th Ed. London, Swan & Sonnenschein & Co., 1900.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Schneider, G.** Ueber den Ersatz von Glas durch Gelatine. *Zeitschr. f. wiss. Mikros. u. f. mikros. Techn.* 18: 288-290, 1902.

In an article on the subject by Dr. Pranter in the preceding number of the *Zeitsch. f. wiss. Mikros.* (reviewed in

*JOURNAL OF APPLIED MICROSCOPY*, 5: 4, 1902), it was stated that thin gelatine plates used for cover-glasses actually possessed all the disadvantages known to belong to the gelatine film. They are soluble in water and other fluids. They bend and melt at a given temperature, as many photographers have learned. These latter observers meet the problem by treating negatives after development and fixation in a dilute formalin solution. This causes the gelatine films to lose their easy solubility, their readiness to melt in, and also their capacity for sticking. The author asks whether such a process may not be applied to the gelatine cover-glass. For two years the author has used with good success the gelatine capsules of the druggist for small glass tubes. Capsules of 25 x 10 mm. were used, these little tubes were filled with 90 per cent. alcohol or a formalin solution, and a label placed in the upper part of the capsule, which was put on as a cover. A small number of such capsules are placed in a receptacle of 70 per cent. alcohol or formalin. Only a few tubes should be used at a time or crushing will result. More than one use of these tubes is impossible, since drying changes their form and often tears them. Experience has shown that not all substances can be equally well carried through in gelatine tubes, yet no general reasons could be discovered for this fact. It would certainly be possible to prepare such capsules when it is especially desired to have thin, transparent walls, and before they are entirely dry to treat them with formalin. Moreover, fine openings may be made in the cover to let out the air on closing the capsule and to admit freely the liquid in which the capsule floats. In many cases it is more convenient to keep the capsule afloat in a certain direction by the enclosed air bubble. Such adaptations are impossible with the customary brittle glass tube. It still remains to be seen whether the formol hardened gelatine is possible for slides, demonstration models, etc.

A. M. C.

**Harris, H. F.** A New Method of Staining Elastic Tissue. *Zeitschr. f. wiss. Mikros. u. f. Mikros. Techn.* 18: 290-291, 1902.

The author accidentally discovered that hematein solutions, prepared in a certain way, have a remarkable affinity for

elastin, giving a clear differentiation of this substance. So satisfactory are the results that the stain was used to the exclusion of the usual Weigert method. The stain is made as follows: Hematoxylin 0.2 g., aluminium chloride 0.1 g., 50 per cent. alcohol 100 c. c. Dissolve the hematoxylin and aluminium chloride

and carefully bring the solution to a boil. Then add carefully 6 gr. mercuric oxid. When the mixture is dark purple remove from flame and cool rapidly. Filter the stain, and add one drop of hydrochloric acid. Sometimes a flocculent precipitate forms a short time after cooling, which dissolves on adding the acid. The stain should be set aside for some weeks, as it does not usually give good results at once; this "ripening" process is variable, but when accomplished, the stain keeps indefinitely. For staining immerse thin sections of tissue in it for 5 to 10 minutes. Wash for 1 minute in 1 per cent. nitric acid in alcohol, remove acid alcohol with pure alcohol, clear, and mount. The author suggests the name *Elasthamatein*, since it is closely related to Mayer's *Muchamatein*. Tissue treated with Mayer's mucicarmine and then with alcoholic solution of nitric acid show the elastin more brilliantly stained than any other structure.

A. M. C.

Michaells, L. Ueber Fettfarbstoffe. Virchow's Arch., 164: 263-270, 1901.

The author starts on a consideration of a selective stain for fats, pomades, paraffin, etc., and chooses alkannin in the form of the extract and a series of azo-stains, among which Sudan III is the best known. This also works well on the living over-fed animal or on the object fixed in formalin. Osmic acid is not exclusive nor inclusive in its affinity for fat, it stains horn substance and only fatty acids. Sudan III has a known definite composition, so that the author has attempted to determine upon what property of the molecule the fat staining propensity depends in order to discover systematically a better fat stain. A number of azo-bodies were made, and the author came to the following results: Fat-stains are those azo-bodies which possess no salt-forming group. He considers them indifferent in contact with acid or basic forms. Scarlet R. (Fettponceau of Kalle & Co., azoorthotoluolazo.— $\beta$ —Naphtol.) proves itself very powerful. This stain is insoluble in water, acids, alkalies, slowly soluble in alcohol, easily soluble in chloroform, fats, oils, and melted paraffin. In concentrated sulphuric acid it loses its blue color and becomes deep red and stains even the smallest fat drop a shining red. Formalin hardened microtome sections or material prepared in other ways is put in a saturated solution of the stain in 60 to 70 per cent. alcohol and stained 15 to 20 minutes. The nuclei may be stained previously by the use of Boehmer's hematoxylin and the mounts made in glycerine or levulose syrup. Glycerine is less clear, but does not affect the hematoxylin. The author shows an interesting theoretical side to the question. No stain shows more clearly that the process by which it acts is a physical and chemical one. The molecule in order to be soluble in fat must be definite in its chemical constitution. The results do not indicate the process to be merely "solution," since the colors to affect the nucleus must be basic, and to give results with the protoplasm must be acid.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Caullery, M. et Mesnil, F. Recherches sur les Orthonectides. Arch. d'Anat. Micros. 4: 381-471, pl. 10-12, 1901.

These parasites, representatives of the so-called *Mesozoa*, were found in the brood-pouches of ophiurans, in the

general cavity of nemerteans, in the digestive cæca of planarians, and in the body cavities of various annelids. The males and females, and in some cases the hermaphrodites, are found in the organs enclosed in so-called *sacs plasmodiaux*, or plasmodes, and very large numbers are usually to be secured from a single host. After removal from the host structural alterations and even disintegration ensue rapidly in sea water. Examination in this medium is, however, necessary for observation of the movements of the plasmodes and the sexual forms and of the ciliation of the latter. Fixation on the slide by osmic vapor followed by picro carmine has been used, as well as fixation and sectioning *in situ* in the host. The authors secured best results by teasing out as evenly as possible upon a cover-slip fragments of the parasitized host. Only a small amount of sea water should be used and the material should not be allowed to dry. The cover-slip is then floated, preparation side down, upon the fixing fluid. Saturated solution of sublimate in sea water to which 1 per cent. acetic acid had been added, gave good results when followed by Mayer's hæmalum. C. A. K.

Laguesse, E. Sur la structure du pancréas chez quelques Ophidiens et particulièrement sur les îlots endocrines. Arch. d'Anat. micros. 4: 157-219, pl. 5, 1901.

To preserve the zymogen granules which Flemming's stronger solution dissolves out of adult tissues, the author

prepares a modification of the mixture in which the osmic acid is increased and the acetic acid much weakened, as in the following formula:

Osmic acid 2 per cent.	-	-	-	4 cm <sup>3</sup>
Chromic acid 1 per cent.	-	-	-	4 or 8 "
Glacial acetic acid	-	-	-	1 drop

Nucleus, cytoplasm and zymogen are well preserved. Tissues should lie in the fluid 24 to 48 hours, be thoroughly washed in running water, and remain in alcohol but a few days before embedding in paraffin. Iron-hæmatoxylin or Flemming's triple stain gave the best results. The latter stain was modified as follows: The sections were stained for 24 hours in anilin-safranin (mixture of equal parts of saturated solution of safranin in absolute alcohol and of water saturated with anilin oil and filtered), after having been mordanted for 24 hours in a saturated solution of potassium sulphate. They are then stained for 24 hours in a gentian-orange mixture prepared as follows: To 3 cm.<sup>3</sup> of an old saturated aqueous solution of gentian violet 3 to 6 drops of a concentrated aqueous solution of orange G is added drop by drop and thoroughly stirred in. Then 3 to 4 cm.<sup>3</sup> of distilled water are added in like manner until the precipi-

tate formed by the mixture is redissolved. After staining, the sections are washed quickly in absolute alcohol and differentiated in clove oil. When the proper differentiation is secured, the clove oil is removed with xylol, and the sections mounted in balsam.

C. A. K.

**Metzner, R.** Untersuchungen an *Megastoma entericum* Grassi aus dem Kaninchendarm. Zeitschr. f. wiss. Zool. 70: 299-320. Taf. 15, 1901.

This peculiar flagellate occurred as a parasite in the intestine of most of the rabbits in the warren of the Physiologi-

cal Institute at Basel, and the author was able to secure abundant material for his investigations. The parasite usually remains attached to the intestinal villi so closely that clean preparations for microscopical examination are secured with difficulty. In one instance a rabbit was examined in which the intestine for a distance of 25 cm. below the pylorus contained only a clear fluid stained with bile, in which immense numbers of the flagellate were found in a free swimming state. From such material it was possible to secure clean preparations. Fresh preparations were examined in the hanging drop, which should be made as flat as possible. Oil immersion lenses can be used on such preparations, but the animals perish very quickly. On account of the rapidity with which abnormal conditions affect this parasite, it is necessary to take many precautions to preserve it. The body of the host should be kept at the normal temperature and the transition from the host to the fixing fluid should be made as quickly as possible. Cover-glass preparations were made as follows: Clean cover-glasses were rubbed with a linen cloth into which a trace of glycerine had been rubbed and a drop of the fluid contents of the intestine carefully flooded on the moistened surface. The cover-glass is then placed preparation side down upon the surface of the fixing fluid. The fluid used was prepared as follows: A 1.5 per cent. solution of sodium chloride is saturated with osmic acid (5.5 parts osmic to 100 salt solution) and to 7 volumes of this is added 1 of a saturated aqueous solution of bichromate of potash (mixture No. 2). Just before using, 2 to 4 drops of fuming nitric acid are added to 12 cm.<sup>3</sup> of this fluid (mixture No. 1). The amount of nitric acid may be increased to 8 to 12 drops. When the preparations are to be fixed the two solutions are placed in watch glasses beneath bell jars. The cover-glass preparations are placed for 2 minutes in mixture No. 1. If 8 drops of the nitric acid have been added to the osmic-bichromate solution, the time may be reduced to 30 seconds; if 12 drops, to 20 seconds. They are then placed for 10 to 20 minutes in No. 2, washed in distilled water and passed through alcohol grades. The preparations were stained in acid-fuchsin for a few minutes at 55° to 60°C. or for 1 to 3 hours at 40° in thermostat. After staining, the preparations were differentiated in picric alcohol of two grades; (1) saturated solution of picric acid in absolute alcohol 1 vol., 20 per cent. alcohol 4 vols.; (2) same ingredients in proportion of 1 to 7. The stronger solution should not act longer than 1½ to 2 minutes, and further differentiation should be controlled under the microscope. Preparations were mounted in xylol-dammar and examined with oil immersion objective and artificial light. For protection of the eyes against fatigue and to bring out the finest details of structure, the author employed a globe filled with a solution of chlorophyll (0.5 cm.<sup>3</sup> of alcoholic chlorophyll extract to 1500 cm.<sup>3</sup> of distilled water) and hung in a circular opening of an asbestos screen. C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Winkler, H.** Ueber Merogonie und Befruchtung. *Jahrb. f. wiss. Bot.* 36: 753-773, 1901.

The author has succeeded in fertilizing enucleated fragments of the egg of *Cystosira barbata*, a representative of the Fucaceæ. Typical segmentation proceeds in such fragments, but at a slower rate than under normal circumstances. The experiments of earlier investigators on the fertilization of enucleated fragments of sea-urchin eggs were repeated. The author devised a very ingenious method of obtaining nucleated and enucleated portions of these eggs. His procedure was as follows: the point of a pipette was drawn out to a capillary and broken off at a point where its diameter was slightly greater than the diameter of an *Echinus* egg. Then across the small opening so made a fine silk or cotton fiber was stretched and fastened to the sides of the capillary with wax or shellac. Then the pipette was filled from above with the water containing the eggs, and the rubber bulb slipped on over the end. With weak pressure the eggs were then forced out through the capillary end of the pipette, being divided into two parts by the cross fiber as they passed out. Of course many of the eggs were crushed and torn, but the operation was so simple and speedy that a large number of clean cut egg fragments containing no part of the nucleus could be obtained in a short time. The author finds this method to be superior in practice to any of the others which have been advocated for the same purpose.

Experiments on sea-urchin eggs were tried in which filtered sperm extract was added to unfertilized eggs. The sperm extract was prepared by violently shaking large quantities of spermatozoa in either distilled water or sea-water, and then subjecting the mixture to a temperature of 70° C. This temperature killed the spermatozoa. The liquid was then filtered and brought to the concentration of sea-water. Segmentation in the unfertilized eggs was induced in some cases as a result of the action of this extract, and the conclusion is reached that the spermatozoa contain a substance (chemical) which induces division in the egg. The final section of the paper is devoted to a theoretical discussion of the phenomenon of fertilization.

R. P.

**Gies, W. J.** Do Spermatozoa contain Enzyme having the Power of Causing Development of Mature Eggs? *Amer. Jour. Physiol.* 6: 53-76, 1901.

An extensive series of experiments in which the effect of extracts of the testes of *Arbacia punctulata* and *Strongylocentrotus purpuratus* on unfertilized eggs of the same species, resulted negatively. No parthenogenetic development was produced. A variety of ordinary methods for enzyme extraction were used and proper precautions were taken against inducing parthenogenesis by change of concentration of the medium. The author's conclusion is that if any enzyme is concerned in the process of fertilization (as seems very doubtful

from these experiments) its action probably is dependent on its being delivered directly to a point within the egg. Winkler's results on the same subject are thought to be due to the effects of osmotic influences.

R. P.

**Loeb, J.** Studies on the Physiological Effects of the Valency and possibly the Electrical Charges of Ions. I.—The Toxic and Antitoxic Effects of Ions as a Function of their Valency and possibly their Electrical Charge. *Amer. Jour. Physiol.* 6:411-433, 1902.

The development of the eggs of *Fun-  
dulus* was used as a test for the toxic and antitoxic effects of chemicals. It was found that the deleterious effect (toxic) of a solution of a single electrolyte on these eggs could be counteracted by the addition of small amounts of some other electrolyte (antitoxic action). For example, no eggs will develop in a  $\frac{5}{8}$  m solution of NaCl, but if to 100 c. c. of this solution, 4 c. c. of an  $\frac{m}{84}$  solution of  $\text{CaSO}_4$  be added, 75 per cent. of the eggs will develop and form embryos. Experiments of this sort were tried with a variety of chemicals and the somewhat remarkable general result was obtained that the marked toxic action of the salts of monovalent kations (Na, Li, K,  $\text{NH}_4$ ) with monovalent anions ( $\text{Cl}$ ,  $\text{NO}_3$ ,  $\text{CH}_3\text{COO}$ ) could be annihilated by the addition of a small amount of salt having a bivalent kation (Ca, Ba, Zn, Bo, Pb). For NaCl, trivalent kations (Al, Cr) have a more energetic antitoxic effect than bivalent kations. Anions of whatever valency were not found to have this antitoxic effect. The author inclines to the view that in the case of NaCl the Cl ion may be the one to which the toxic effect is due, although in previous papers he has advocated the opposite position. From the fact that in a series of sodium salts the toxicity increases with the increase in the valency in the anion, Loeb concludes that the poisonous effects of salts with a univalent kation are due to the negative charges (or negative electrons) of the anions.

In concluding, the author develops the view that the electrical charges of ions produce their effect on life phenomena by changing the physical condition (coagulating or liquefying) of the colloidal material of which protoplasm is in the main composed.

R. P.

**Loeb, J. and Lewis, W. H.** On the Prolongation of the Life of the Unfertilized Egg of Sea-Urchins by Potassium Cyanide. *Amer. Jour. Physiol.* 6: 305-317, 1902.

Unfertilized eggs of the sea-urchin lose their power of developing in about twenty-four hours if they are left in ordinary sea water after deposition.

The authors of this paper found that by subjecting these unfertilized eggs to the action of a mixture of about 100 parts of sea water and one part of an  $\frac{n}{10}$  KCN solution for a varying length of time their power of development could be preserved for a much greater period than when they were left in the sea water alone. The matured but unfertilized eggs were taken from the animals and placed in the KCN solution. After they had remained for some time in this solution they were transferred to ordinary sea water and fertilized. It was found that by gradually diminishing the strength of the KCN as the experiment progressed it was possible to obtain plutei from eggs which had been subjected to the action of this solution for as long as 112 hours. It was also possible to induce parthenogenetic development in eggs which had been subjected to the action of KCN in a similar manner. The authors believe that there are going on in the unfertilized egg "specific mortal processes," which, unchecked, lead to its rather speedy dissolution. Normally fertilization checks or modifies these processes. In the experiments described in this paper these processes are checked by the poison KCN. It is to be regretted that the authors chose to designate as "prolongation of life" a process which is evidently, as they themselves admit, nothing more than a suspension of vital activities for a certain time ("vie latente"). Such phenomena are, of course, well known in other cases.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Locke and Cabot.** Iodophilia. *J. of Med. Research*, 2: 25-42, 1902.

Locke and Cabot in a preliminary report from the Massachusetts General

Hospital (*The Journal of Medical Research*, January, 1902) discuss the subject of iodophilia. This is the reaction which certain of the white cells of the blood show when a dried blood-film is brought in contact with the following solution:

Iodine,	-	-	-	-	-	-	1 gram.
Potassium Iodide,	-	-	-	-	-	-	3 grams.
Water,	-	-	-	-	-	-	100 c. c.
Gum arabic,	-	-	-	-	-	-	50 grams.

This mixture was first suggested by Ehrlich, though the reaction had been long known. The technique consists in a cover-glass film being prepared in any of the usual ways and allowed to dry in the air. Without any fixation it is then pressed down upon a drop of the iodine solution on a slide and examined with an oil immersion lens. If the blood be normal the red cells are uniformly colored a bright yellow upon a much fainter background, while the white corpuscles are stained of about the same tint, their nuclei being somewhat more refractile.

In certain pathological conditions the iodine reaction is noted. This consists in the uniform yellow coloration being broken by the appearance in the protoplasm of the polymorphonuclear neutrophiles of reddish brown granules, or a diffuse brownish coloration, and by the presence of small and large masses outside the corpuscles similarly colored. The reaction then may be extra-cellular or intra-cellular. In rare cases basophiles and myelocytes react. In their examinations, as a routine, at least one hundred cells were counted; if in that number none were observed with either a diffuse or granular stain they considered the reaction negative. The history of the reaction is briefly given. The observations of the different observers have been quite varied. Locke and Cabot studied the reaction in 482 cases. They give a table of their results from such a study and follow it with brief histories of eight cases to show the practical application of this reaction in differential diagnosis.

They conclude that the increase of extra-cellular bodies is of little significance. Intra-cellular granules, however, are never found in normal blood and they believe them to be pathological. In regard to iodophilias, they assert:

(1) It signifies not a special disease or condition, but a general toxemia. The diagnosis of pus, however, should not be made from this condition alone.

(2) It is not identical with, neither does it coincide in its indications with any of the ordinary physical signs, as leucocytosis, fever, etc.

(3) It appears to be certain evidence that the patient is sick. It is a more reliable sign than either leucocytosis or fever.

(4) A positive reaction occurs with considerable regularity in the following conditions:

- a. Infection with pyogenic organisms, either local or general.
- b. Toxemia of bacterial origin, as in diphtheria and typhoid.
- c. Non-bacterial toxemia: e. g., uremia.
- d. Disturbances of respiration.
- e. Grave anemia, both primary and secondary.

(5) The sign is absent, in their experience, in pleurisy, rheumatism, extra-uterine pregnancy, alcoholism, abscesses with free drainage, lead poisoning, early malignant disease, nervous conditions, tuberculosis if uncomplicated by secondary infection, and various other diseases.

W. R. S.

**Wright, J. H.** A Case of Multiple Myeloma. Contributions to the Science of Medicine, dedicated by his pupils to Dr. W. H. Welch. Balto., pp. 359-366, 1900.

The following interesting pathological report is given by Wright on a patient with multiple myeloma associated with albumosuria. Tumor masses, mostly

small in size, were found in the sternum, ribs, vertebræ and skull. On section they appeared generally to consist of soft, mushy, red tissue, somewhat resembling bone marrow. Microscopically these masses were composed of small cells closely crowded together. Interspersed among them were rather numerous thin-walled blood vessels. At first glance there seemed to be no supporting framework, but on closer inspection a few delicate fibrillæ were revealed. The cells were somewhat variable in size, circular in outline and with nuclei of a round or slightly oval shape. These nuclei varied in diameter, but were usually one-half or two-thirds the size of the cell. They were often eccentrically placed. A striking characteristic of these nuclei was the presence of a variable number of intensely chromatin staining masses, of round or oval outline, continuous with the nuclear membrane and projecting from it into the interior of the nucleus. There was also a well marked, round globule of chromatin on the center of the nucleus. Some of the cells had two nuclei and very few had three. The cytoplasm was homogenous.

Wright thinks these cells are more closely related to plasma cells than to marrow cells, although their cytoplasm did not in all cases show a marked affinity for methylene blue. The new growth consequently could be spoken of as a plasmoma.

W. R. S.

**Watanabe.** Versuche über die Wirkung in die Trachea eingeführter Tuberkelbacillen auf die Lunge von Kaninchen. Ziegler's Beiträge, Bd. 31, pp. 366-382, 1902.

Watanabe reports the results of his work on injecting tubercle bacilli into the trachea of dogs. They were killed from twelve hours to sixteen days after

the injection. The first injury he found was in the smallest bronchioles and in the alveoli. In these places the epithelial cells were swollen and desquamated. At the same time a serous exudation occurred and a migration of polynuclear leucocytes. In other words, we have here an example of a catarrhal inflammation. The growth of connective tissue occurred secondarily and was accompanied by a grouping together of mononuclear leucocytes. A decrease in the number of polynuclear leucocytes was then observed. Giant cells appeared to be formed by the fusion of several cells.

W. R. S.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

**Pakes.** On the Value of Plating as a Means of Determining the Number of Bacteria in Drinking Water. *Cent. f. Bac.* II, 7: 386.

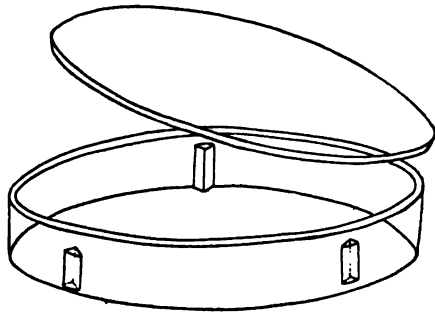
The author has attempted to test the value of the ordinary methods of studying water in determining the actual number of bacteria, and reaches some rather unexpected conclusions. He finds, in the first place, that the ordinary nutrient gelatin is not a favorable medium for bacteria growth. He finds, indeed, that a medium made by dissolving gelatin in distilled water and then neutralizing with NaOH without the addition of meat extract or gelatin is the medium in which the water bacteria apparently grow the best, for such gelatin give larger numbers than the ordinary nutrient gelatin. He then tests nutrient material made of gelatin dissolved in waters and finds wide differences in the number of bacteria which grow in the different samples of gelatin dissolved in water from different sources. The great irregularity leads him to believe that in order that the largest number of bacteria possible should be obtained the culture media should be as nearly as possible like that of the water tested. He concludes in general that the use of ordinary gelatin gives no necessary criterion of the number of bacteria present in water. Sometimes, indeed, the results are widely out of harmony with the actual numbers. He recommends that the gelatin culture medium to be used should be made without meat extractives and should be made with the water which is to be examined, and claims that the best nutrient material is that of pure water with gelatin and without any further additions, except for neutralization.

H. W. C.

**Turro.** Zur Anärobenkultur. *Cent. f. Bac.* I, 31: 175.

A simple, practical device for the isolation of anærobic bacteria has been described by the author, and consists of the following:

The apparatus used is shown in the accompanying figure. It consists of a common Petri dish with glass rests fastened upon the inner side, and a glass disk which just fits into the Petri dish, resting upon the glass supports. For use the gelatin culture medium is heated in an autoclav to drive out all the air. It is then poured upon the glass disk and inoculated. After it has hardened there is placed in the dish a quantity of pyrogallic acid and NaOH, and then the disk, with its inoculated gelatin, is placed upon the rests, with the gelatin side down. Melted paraffin is poured around



the edges of the disk to make a tight seal and prevent the entrance of air. The pyrogallic acid soon absorbs the oxygen in the Petri dish, and the anærobic organisms grow readily, and can be easily studied under a microscope. The author also describes a special form of tube for cultivating anærobic bacteria.

H. W. C.

**Blot.** A New Method of Intensive Coloration of the Bacillus of Koch. Report read at the third session of the Association of Anatomists, Lyons, 1901.

The most satisfactory methods of staining the bacillus of Koch are those of Ehrlich and of Ziehl; even these present difficulties which, however, the

author has at last succeeded in overcoming. In Ehrlich's method the different solutions used are made with difficulty; they require a long time for their preparation, they cannot be procured easily and they do not keep. Ziehl's method is very good, and very rapid, but has the slight drawback of giving the bacilli a more or less decided red tint, and of being hard to manage because the manipulator must know how to arrest the process of decoloration at precisely the right moment. This moment, in itself, is difficult to determine, because the bacilli which may already have become decolorized, are no longer visible.

The method suggested by the author, being certain and expeditious, and eliminating all possibility of error, will be of great value to practitioners. It is as follows:

Prepare the specimens in the usual way—that is, crush a minute fragment of the substance to be studied between two cover-glasses, which should be separated almost immediately. Allow them to dry, heat them (in order to insure the coagulation of albuminoid material) by passing them three times through the flame of a Bunsen burner or an alcohol lamp. Into a small porcelain capsule pour 5 to 6 c. c. of water slightly carbolated (2 to 3 parts per 1000), add to it 8 to 10 drops of a saturated alcoholic solution of fuchsin, warm gently until the first bubbles appear, plunge the cover-glass for an instant into a 20 per cent. solution of nitric acid, dip it slowly into the alcohol until the decoloration is as complete as possible (methylated alcohol may be used), wash, and then immerse in a watch-glass containing 2 to 3 c. c. of a 40 per cent. commercial solution of formic aldehyde. The stain of the bacilli will be more or less dark, depending upon the concentration of the aldehyde and the length of time the cover-glass is immersed in it. Two to four minutes is usually sufficient.

The decoloration should be pushed as far as possible in order that, during the immersion in the aldehyde, the cellular elements which constitute the background may not regain their stain. Afterward wash in pure water, and examine in a drop of water. If the stain is not deep enough, immerse the cover-glass again in the aldehyde for a longer or shorter time. After washing with water, the specimen may be dried and mounted in Canada balsam. The bacilli can be seen in a deep stain which appears to be black, but which is in reality a blackish violet. It is not necessary to counter-stain the background in order to differentiate the bacilli from the other elements.

A. GIRAULD.

Translated by Eleanor L. Lattimore.

## NEWS AND NOTES.

The eighth annual session of the Biological Station of the University of Indiana will be held at Winona Lake, Kosciusko county, Indiana, between June 23 and August 22. The station is a field laboratory and especial emphasis is laid on field work. In general the work will be adapted to the needs of medical preparatory students and of teachers and investigators in the lines of Zoölogy, Physiology, Botany and Nature Study.

The Harpswell Laboratory of Tufts College located at South Harpswell, on Casco Bay, which is sixteen miles from Portland, Maine, will be open from June 16 to September 13, 1902; the regular courses of instruction beginning July 2 and continuing for six weeks. Courses will be given in Intermediate Zoölogy, Vertebrate Zoölogy, Botany and Embryology. The laboratory also provides for opportunities for original research. Communications concerning the laboratory should be addressed to the director, J. S. Kingsley, Tufts College, Mass.

The University of Montana Biological Station will open July 14, and continue five weeks, or until August 16. Courses in Zoölogy, Botany and Nature Study will be given and ample opportunity for original work in the unexplored regions surrounding the station. Information concerning the station may be obtained from Morton J. Elrod, Missoula, Montana.

The eleventh session of the Hopkins Seaside Laboratory of Leland Stanford Jr. University will begin June 9, 1902, and continue six weeks, closing July 19. The laboratory provides for three classes of students: Teachers and students, for whom regular laboratory courses in Botany and Zoölogy are arranged; advanced students in Zoölogy, Physiology, and Botany; and investigators who are prepared to carry on researches in Morphology and Physiology.

**METHODS IN THE CULTURE OF UNICELLULAR ALGÆ.**—Knop's solution either as a fluid culture or in connection with agar (5 to 7 grams to the liter of nutrient solution) gives very satisfactory results. Stender dishes serve well for culture vessels, as it is desirable to have as large an amount of the medium as possible and also considerable surface for growth. Sterilization must be as complete as in bacteriological work. It is found better to deposit the algal cells on the moisture that collects upon the surface of the agar after sterilization than to place them directly upon the agar, as in the latter case growth is retarded; but when deposited in the moisture, which slowly evaporates, the contact with the agar is gradual and growth proceeds normally.

When it is desired to make direct and frequent examination of cultures a very satisfactory method is to isolate a single cell on a slide in sterilized water or nutrient solution and place over it a cover-glass supported by wax feet. Evap-

oration may be prevented by placing over the mount a bell-jar lined with moist filter paper.—*Bot. Gaz.* 32: 5.

In a report to the U. S. Department of Agriculture, Mr. W. R. Beattie describes a plan for freeing a botanical laboratory from a plague of cockroaches or other insect pests. The agent used is hydro-cyanic acid gas, liberated by the action of dilute sulphuric acid on potassium cyanide, about 1.5 gr. of the latter being used per cubic foot of the building. The sulphuric acid is placed in glass jars, above each of which is fixed a pulley. A line passing over this pulley carries the packet of cyanide, which may thus be lowered into the acid from the exterior of the building. All outside openings are closed as far as possible and the cyanide being lowered into the acid, the building is left to itself for at least three hours. The insects leave their hiding places as the fumes reach them and die on the floor where they may be swept up.—*Eng. Mech. and W. of Sci.* 74: 1901.

**HATAI'S FLUIDS FOR FIXING SPINAL GANGLION CELLS.**—Hatai studied the ganglion cells of the rat and found the following solutions more satisfactory than any used, among which were Carnoy's, Ewing's, Leuhosseck's, Graf's and Gilson's fluids: .

- |   |          |
|---|----------|
| I. Corrosive sublimate, sat. sol. in formalin       | 6 parts  |
| Glacial acetic acid . . . . .                       | 10 parts |
| Normal salt solution . . . . .                      | 3 parts  |
| II. Picric acid, sat. sol. in 10 per cent. formalin | 3 parts  |
| Glacial acetic acid . . . . .                       | 1 part   |
| Corrosive sublimate . . . . .                       | 1 part   |

In using either of these fluids the following procedure is recommended:

1. Allow to remain in solution six to twelve hours.
2. Wash in running water four to five hours.
3. Wash in weak alcohol (30 per cent.), after which the usual paraffin embedding method is pursued.

In staining after this fixative, thionin, toluidin blue, and methylen blue gave best results.—*Jour. Comp. Neurology*, 11: 1.

Through an oversight the proper heading for the review on page 1771 of last month's JOURNAL was omitted, leaving the reader in doubt as to the source of the matter reviewed, the subject of the review, which began in the February number, being Vol. XXII of the Transactions of the American Microscopical Society.

### Books Received.

**Annual Report of the Smithsonian Institution, 1899.** U. S. National Museum.

**The Etiology of Yellow Fever.** Reed, Carroll, and Agramonte.

**Transactions of the American Microscopical Society, Vol. XXII.**

**Twelfth Annual Report of the Missouri Botanical Garden.**

**Flowers and Ferns in Their Haunts.** Mabel Osgood Wright.

**Methods in Plant Histology.** Charles J. Chamberlain.

**Index Catalogue of the Library of the Surgeon-General's Office, U. S. Army.** Second Series, Vol. VI. This volume includes 15,589 author's titles, representing 5,865 volumes and 14,296 pamphlets. It also contains 5,962 subject titles of separate books and pamphlets, and 30,561 titles of articles in periodicals. The library now contains 138,078 bound volumes and 235,127 pamphlets.

# Journal of **Applied Microscopy** and **Laboratory Methods**

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## **The New Laboratory for Plant Physiology of the Agricultural Academy in Poppelsdorf-Bonn.**

Two years ago there was erected in Bonn, Germany, the seat of botanical learning made renowned by the labors of Hanstein, Sachs and Strasburger, a laboratory for plant physiology of the most modern type. Through the courtesy of Prof. Dr. Fritz Noll, the Director of the Agricultural Academy, of whose prac-

FIG. 1.

tical ideas the building is an embodiment, the writer is able to present an account of the same.

The building is a low one-storied structure with a basement, constructed of brick in solid German fashion, and externally presents a somewhat unusual

(1929)

FIG. 2.

appearance due to the simple but unique circumstance that its architectural features have been determined by, and made subservient to, the object for which it was constructed. Laying aside the question of appearances, concerning which there would be, no doubt, a considerable difference of opinion, we must congratulate Professor Noll on his success in gaining his object, success which, I am

FIG. 3.

assured, was gained only after the persistent vigilance and oversight which he personally gave it.

The structure stands in the rear of the main laboratory building of the Agricultural School, and on the border of the garden which contains a large collection of economic plants, and which was made famous by the researches of Professor Friedrich Koernicke upon the varieties of the grains. A part of this garden appears in the foreground of our illustration, Fig. 1, in which the northerly elevation of the building appears, showing the glass roof and wall of the main laboratory for research in plant physiology. In the southerly elevation, shown in Fig. 2, the ventilating windows of this laboratory are to be seen, while to the

FIG. 4.

left are the external details of a unique feature of the plant, the terrarium or "Wurzeltunnel," as it is called upon the ground, to be described beyond.

The total length of the building is 20.26 meters, with a depth of 8.88 meters. Interiorly it is broken up into two series of rooms (see Fig. 8) which we pass on to examine in turn. Under the guidance of Professor Noll, we should probably enter the room for microscopy, at the northeast corner, occupied by him as an office. Here we find a cupboard stocked with the more delicate instruments of precision for physiological work and a small but efficient working collection of physiological books, beginning with the earliest classics.

Passing into the large, glass roofed laboratory, we find ourselves in a splendidly equipped place, as the reader may judge by examining the interior views, Figs. 3 and 4. Along the open side of this laboratory are broad cement tables, so constructed as to be interchangeable from glass topped tables to cement cul-

ture beds. For the reception of water cultures, and potted plants under experiment, seen in use in this way in Fig. 4, the heavy glass tops may be used. By removing the glass tops the tables are then in the form of shallow tanks, to receive a bed of sand, or soil, as the case may require, to the depth or some 15 or 20 cm. Being constructed of concrete, the whole is thoroughly solid, and does not warp or sour. A smaller table, of similar construction, is found at either end of the room. In Fig. 3, this table is occupied by salt water aquaria, in some of which at the time of our visit a fine growth of a *Bryopsis* was being cultivated for experimental purposes. On the corresponding table at the other end of the room, one may see, in Fig. 4, three double walled bottles for experiment with different colored lights. It will be noticed that they are not uniform

FIG. 3.

in size or shape, although made at the same time and place, from which circumstance it may be inferred that they are blown and not moulded. These three bottles are of unusual historic interest to botanists, since they are those used by Sachs in his first work on the effects upon growth of the different parts of the spectrum. They are still sound, and in every day use!

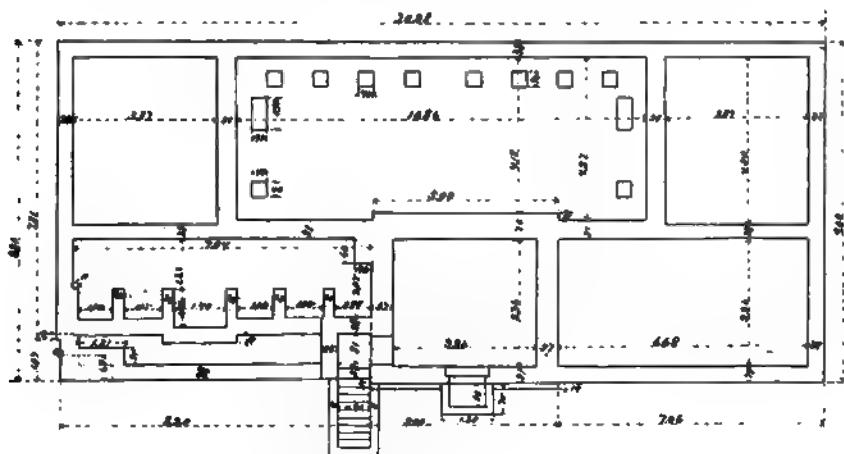
In two corners stand iron tanks, each of about a cubic meter capacity, for holding a supply of rain and of distilled water (Fig. 4). In a third corner there is built in a moist chamber (Fig. 3), constructed of iron, for the framing, and glass. The walls are lined with a rubblework, made by setting small pieces of pumice into cement. The result is a rough surface holding the water, which is supplied from a perforated pipe running around the top of the chamber. If necessary, the flow of water over the walls may be made continual. The floor is of

**FIG. 8.**

cement. The remaining wall space of the laboratory is occupied by shelving and racks for apparatus, glassware, tools and the like.

In the center of the room stands, upon the floor, a cement tank (Figs. 3 and 4), into which open several taps to which may be attached aspirators, or water motors for keeping clinostats in motion. Behind the tank is a potting table supplied with convenient bins for holding clean sand and soil, and a fine spray-rose for moistening the soil as needed.

The lighting of the room, an important desideratum, is modified at pleasure by means of shades of jute bunting, which allows the direct sunlight to pass through, but at the same time considerably lowers the amount.



**FIG. 7.—Foundation Plan.**

At the west end of the main laboratory one enters a dark room of liberal dimensions which may be used for photographic purposes, as well as physiological. In this room also there is a moist chamber, fashioned like the one described above.

From the dark room a door leads to the terrarium or "Wurzetunnel," a portion of which appears in our reproduction, Fig. 5. The purpose of this feature is to make possible the careful study of economic plants in relation to the soil, especially regarding the problems of nutrition. The plan of the terrarium makes it possible to grow the plants under investigation out of doors, and yet have the soil and watering completely under control. This is accomplished in the follow-

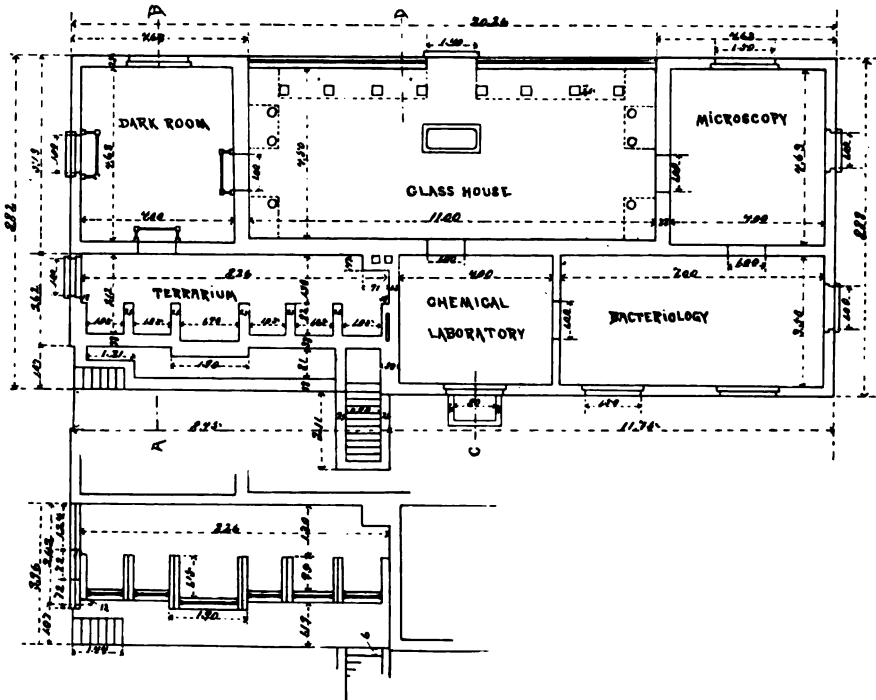


FIG. 8.—Floor Plan.

ing manner. Along one side of the terrarium are six tanks built of brick and cement. The sides toward the room are of plate glass, and are, with one exception, placed in sloping position; this the better to study the behavior of the root systems. To guard against phototropic reactions, the roof of the terrarium is made of yellow glass, which, therefore, admits only inert light in this regard. Ventilation is had by means of a ventilator, which admits no light, in the door. Taps at the tank bottoms, which have a proper slope, allow the water to be drawn off for analysis if it is so desired.

Externally the compartments are made easy of access by sliding backs, which may be removed when it is desired to work at the cultures. The arrangement as it appears is shown in Fig. 6.

The remaining rooms in the building are quite simple, and are fitted up for chemical and bacteriological work and call for no special mention, beyond the statement, which applies to the laboratory as a whole, that there is an abundance of simple but thoroughly efficient apparatus—tools, one

would perhaps better say—and that one can obtain gas or water at almost any point desired.

FIG. 9.—Section through terrarium.

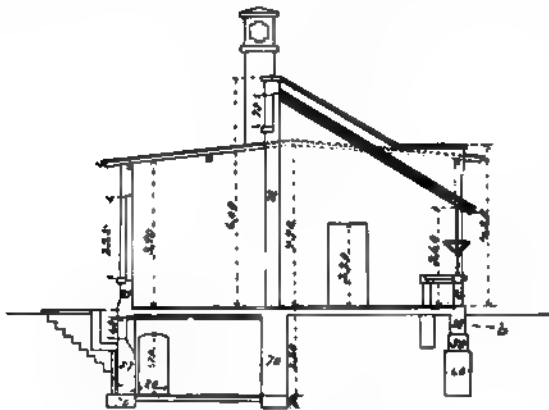


FIG. 10.—Section through glass house.

The writer is under the belief that this admirable little laboratory possesses many points of interest and importance to those concerned in economic experimental work in agriculture and horticulture, as well as to plant physiologists in general.

The half-tone illustrations are from photographs made by the writer. The plans given herewith are taken from

a blue print of the originals most kindly furnished by Professor Noll, to whose

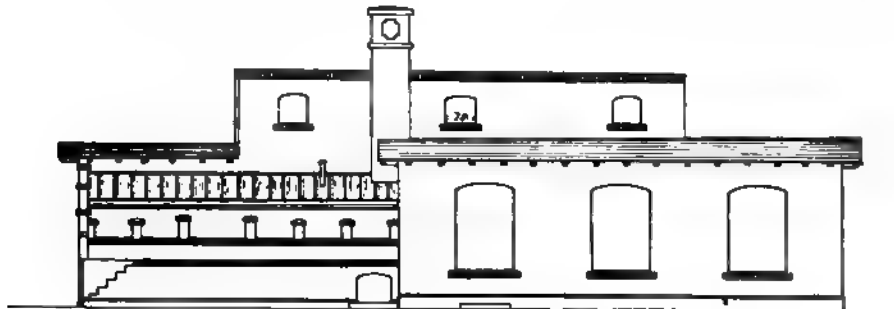


FIG. 11.—Southerly elevation.

very great courtesy and warm-hearted disinterestedness in this and other ways the writer is greatly indebted.

Teachers College, Columbia University.

F. E. LLOYD.

### An Improved Method for Staining Flagella.

Up to the present time no method has been devised that will enable one to stain flagella with any degree of certainty.

The great difficulty—according to the writer's experience—has been in the amount of handling necessary to secure the proper dilution of bacteria upon the cover-glass.

This can be done with very little handling by the following method: to a tube containing 5 c. c. of sterile water, add enough of an 18–24 hour agar culture of bacteria to produce a very faint turbidity in the upper half of the water.

After the proper amount of bacteria have been added, the tube is placed in an incubator run at the optimum temperature for the particular species under consideration, and incubated for one hour. After the culture has been incubated, two or three drops of the culture are placed upon a clean cover-glass and allowed to dry spontaneously at the temperature of incubation. Fix by passing through the flame, and stain by Pitfield's flagella stain.

#### PITFIELD'S FLAGELLA STAIN.

##### *The Mordant:*

Tannic acid, 10 per cent. aqueous solution	-	-	10 c. c.
Corrosive sublimate, saturated aqueous solution	-	-	5 c. c.
Alum, saturated aqueous solution	-	-	5 c. c.
Carbol fuchsin	-	-	5 c. c.

##### *The Stain:*

Alum, saturated aqueous solution	-	-	10 c. c.
Gentian-violet, saturated alcoholic solution	-	-	2 c. c.

Pour over the dried and fixed film as much of the mordant as it will hold. Heat over the free flame until steam begins to rise; keep at this temperature for one minute. Wash in running water, dry thoroughly, and add stain. Heat, dry, and mount in balsam. This method has given very satisfactory results in the hands of the writer and his colleagues.

ARTHUR I. KENDALL.

Lawrence, Mass.

### On Preparing Vertebrate Skeletons.

A collection of the skeletons of the different familiar vertebrates is of great practical value to any biological department, but their cost if purchased outright or the supposed difficulty of preparation brings it to pass that although a collection of mounted skins has much less teaching value and costs more, it is more common to see a collection of mounted animals, especially of birds, than a collection of skeletons. The processes of skeletonizing are not as difficult as perhaps they may seem, and less exacting than those of zoölogical technique in many other departments. If they were more familiar it would often be possible to save materials for osteological preparations which at present are thrown out with the waste. There are frequently students in a biological department pos-

sessed of sufficient mechanical skill to make good preparations with the aid of a few suggestions and a little oversight at the outset. And the practice is also one of the best ways of gaining ground in the science of osteology, of so much interest in the comparative anatomy of the vertebrates. The present article is a statement of the experiences, chiefly those of Mr. Tyrrell, a student in Hamline University, who has successfully prepared a considerable number of skeletons of both birds and mammals. It is presupposed that the reader will have access to one or more mounted skeletons of some good firm, for example, that of H. A. Ward of Rochester, New York. Two of Mr. Tyrrell's skeletons are shown in Figs. 1 and 2. The first is a porcupine from the Philippine Islands. It was a rough dried skeleton when he took hold of it. The other is a sea gull. Both are "articulated skeletons," that is, the natural ligaments still hold most of the bones together.

FIG. 1.—Skeleton of Philippine Porcupine.

The first step, after cataloguing the subject and attaching a metal tag with the number stamped on it to a convenient part, is to remove the skin. This is usually done completely, that is, to the tips of the fingers, from the head, etc. We have, however, in some cases departed a little from the traditions at this point and retain the skin on one of the front and one of the hind feet of mammals and on the leg and foot of a bird, and the wing feathers as well. This shows in Fig. 2; in that instance the skin of the tarsus, the webbing of the toes, and the relation of the primary and secondary feathers of the wing to the bone of the arm are shown, thereby adding much to the value of the preparation for the purposes of instruction. After the removal of the skin, the viscera are to be removed and the inner walls of the abdominal and thoracic cavities picked moderately clean. A pair of surgical forceps is used to reach the deeper places.

The eyes, tongue, throat, œsophagus and trachea must be cut away and the brain removed, or the latter may be left to macerate if one is in a hurry. Sometimes these parts may some of them be worth saving as moist preparations in alcohol or formalin for the anatomical museum. If it is desirable to save the brain the roof of the skull must be removed by cutting around its edges and the brain taken out; the roof can later be restored to its place. All the muscular tissue is next to be taken off. It is best to remove the larger limb muscles one at a time, loosening them, running out to the tendons and then cutting the tendon at its attachment to the bone. In this way you run very little risk of cutting the ligaments of the joints, on whose integrity everything will depend. After the larger muscles are taken off the muscles of the spinal column and the intercostals should be cut away to some extent, but this need not be carried very far, as the maceration will take care of them in an easier way. If you are not interested in keeping the skin structures the entire frame can now be macerated, but in case you care to preserve them the limbs should be disarticulated and treated separately.

The skeleton must not be boiled, beautiful disarticulated skeletons can be readily made by boiling, but for a smaller animal there is no practicable way of subsequently mounting them. By macerating in water the muscular tissue is decomposed and can be removed before the more obstinate connective tissues are influenced. Advantage is taken of this fact, and the whole secret of preparing skeletons for mounting lies just here. We have no difficulty whatever in making skeletons with very little trouble by giving close attention at this point. The soaking must be done in a stone or glass vessel. We found that tin or iron ones blackened the bones, though galvanized iron was used without detriment. The water may be changed occasionally during this stage, but it is not necessary. It would be an improvement if the maceration could be done in running water, but this is not economical in our laboratory at least (as we have a water meter). There is no rule as to the time required for maceration, it depends on so many circumstances. The preparation must be taken out from time to time and examined as to the softness of the muscle tissue. When it is soft enough to be easily removed it is time to take it out. The intervals we have noted range from six days to six weeks; the latter, however, in the case of rough dried material. It is hardly necessary to remark that the chemical reactions taking place during the maceration process are some of them accompanied by the liberation of volatile products considered by some to be offensive to the sense of smell. It is accordingly advisable to conduct this part of the process in a room that can be kept shut off from the general laboratory. With a good draught these odors are soon dissipated; there is of course no way to eliminate them from the process.

When the maceration is completed the skeleton is held under a tap and well washed by a strong stream of water. If necessary some of the undecomposed meat may be cut off and the maceration may proceed further, if on examination it is found that the deeper masses of flesh are not sufficiently decomposed. The skeleton at this point will look more ragged than it will after drying, for in drying there is a surprising amount of shrinkage of the softer parts; the bleaching, too, removes some of the last traces of muscle, so that complete cleaning at this

time is not strictly a condition of success. The washing should be supplemented by some scrubbing of the spinal column and thorax with a brush (old tooth brushes are "just the thing" for this with moderate sized animals). This will reach into corners and get out residues that resist the stream of running water.

After the washing has been done as thoroughly as you deem necessary the skeleton can be put to soak in formalin; we have used a one-half to one per cent. solution. This deodorizes and preserves. It enables you to keep the skeleton till later in case you haven't time to go on. We have found it very inadvisable to let the skeleton dry at this point, or indeed at any time till it is completed, if it

FIG. 2.—Skeleton of Sea Gull.

can possibly be avoided. The ligaments never regain the condition they are in at the close of maceration, and this interferes later with "setting up" the frame.

A word about "rough drying" will be in place here. One often comes into the possession of good material for osteology when he is away from home. In that case what can be done? Figure 2 is a skeleton made from such a case. The gull was given to me at a camp where I hadn't the means for preparing it. I removed the skin, viscera and most of the muscles and exposed the frame to the warm air. It soon dried without becoming putrid. If not enough flesh is cut away the frame will putrefy, including the ligaments at the joints. After my return to Saint Paul Mr. Tyrrell took the rough dried skeleton, removed the

wings and legs and put the rest to soak. It took longer to soften the meat and decay it, but in time the skeleton took the form you see. It was, however, impossible to get the bones whitened by any of the devices that we employed.

The wings or legs that are to be preserved with the outer skin structures showing are carefully cleaned by dissection and then by bleaching; it is a tedious process to do them in that way. In cases where epidermal tissues or remnants of connective tissue or muscle are left in any part of the skeleton they invite the attacks of moths and other museum pests. Mr. Tyrrell has found that shellac dissolved in alcohol spread on the skin coats it with a layer which excludes these offenders. We have not experimented on this point, but should think that a weak solution of corrosive sublimate such as is used by the botanists in poisoning plants could be used to advantage.

If a skeleton is made very soon after the animal has died, it will come out nearly or quite white. We have a very beautiful parrot skeleton that is as white as ivory without having been bleached, but in many instances a treatment with a bleaching solution if very carefully conducted will be well repaid. We owe the formula we have used to Mr. H. W. Howling, a taxidermist of Minneapolis. It consists of one-half pound of quick-lime, one pound of sal soda dissolved in one gallon of water. This solution is too strong for any but the heaviest bones or skulls, and for such skeletons as we have been describing must be reduced with at least half its weight of water. It is raised to the boiling point and the skeleton is immersed in it and carefully watched; a little too long, and the bones will fall apart, but less time than this and they are beautifully whitened. The skeleton must be removed and held under the tap and thoroughly rinsed to remove the alkali. It is found, too, that the last traces of flesh have been eaten by the bleacher and the skeleton left in a beautiful condition. It must not be allowed to dry now till it is in final position. If you haven't time to adjust it put it in weak formalin, which is a most convenient fluid for holding things *in statu quo*.

In the final setting up of the skeleton much depends on the "naturalness" of the position of the different parts. This must first be learned by having recourse to good pictures of the animals from life, or, better, if the living animal can be seen by a direct study from that source. The hind legs if they have been separated are attached by drilling with a small hand-drill a hole through them and through the acetabulum and running a piece of soft wire through and bending the ends. If you do not know about tools tell a good mechanic your needs and let him select a hand drill for you and a pair of pliers for twisting wire. A good small drill can be made by mounting a needle in a wooden handle and grinding the end off. Several grades of brass wire are needed; the spring wire is best. It can be heated and thus used for soft wire too. The first step in the final stage of the process is to select a piece of wire of approximately the size of the spinal canal and to give it all the bends that the spinal column of the animal would have in life. These give the shape to the body. The wire is then inserted and pushed into position. A piece is left sticking out anteriorly, to which a piece of cork is fitted the size of the foramen magnum, to attach the head. If the object is a mammal or other quadruped, it is now supported by means of

two wire posts, one in front and one behind. These are made by sawing a slit in the end of a piece of wire and then opening it to form a fork, which is securely bent around the vertebral column. The lower ends of these posts are then set into a temporary pine base and let down a sufficient distance to bring the front and hind limbs into a correct position. The limbs are then adjusted and securely held by means of pins. The skull is not put on till later. The whole is then set aside in a clean place to dry, after which it can be transferred to a permanent stand of black-walnut or such other wood as may be preferred. In case of a larger mammal such as a dog or sheep, an iron rod of specified dimensions should be made at a blacksmith's; the necessary bends can be put in it when you are ready to mount, unless it should be too large an animal.

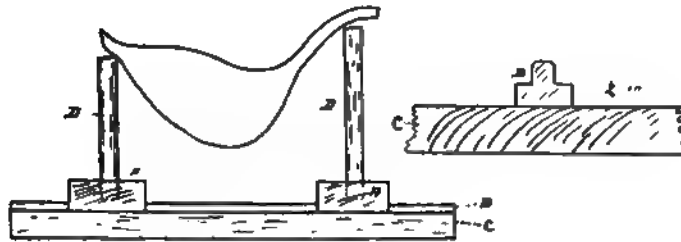


FIG. 3.

Where the legs have been disarticulated in a mammal or a bird the body can be dried on a frame which can be cheaply made as indicated in Fig. 3 by cutting off the tongue and groove of a piece of matched board and using them to adjust the distance apart of two uprights fixed to them which swing the axial skeleton and ribs as in the diagram. The parts are pressed into position and held there if necessary with wires until they dry in the final shape, after which the legs are attached. A post is placed under the keel of the sternum, as in Fig. 2.

Heads are soaked and washed and bleached like the rest. If the lower jaw does not adhere it may be wired to the rest. The head is fitted over a piece of cork attached to the end of the "spinal wire"; lighter heads are sufficiently supported by this mode, but heavier ones may need a wire to run from the occipital bone backward to one of the cervical neural spines, as in Fig. 1.

H. L. OSBORN.

C. C. TYRRELL.

Biological Laboratory, Hamline University.

**LABORATORY PHOTOGRAPHY.***Devoted to Methods and Apparatus for Converting an Object into an Illustration.***SOME SUGGESTIONS ON ONE WAY TO MAKE LANTERN SLIDES.**

If one must reduce the size of his picture so as to get it on a plate so small as a lantern slide plate ( $3\frac{1}{4} \times 4\frac{1}{4}$ ), he must have an apparatus similar to the one shown in the cut, that is, if he wishes to make a slide from a negative of any size from  $4 \times 5$  to  $8 \times 10$ , or larger. If he is to make them from negatives of various sizes he must have a series of kits, the one fitting into the other, similar to 1, 2, 3, and 4 in the cut. (1) holds a  $3\frac{1}{4} \times 4\frac{1}{4}$  and fits into the  $4 \times 5$  opening

**Apparatus for making Lantern Slides.**

of (2), which carries a  $4 \times 5$  plate and fits into the opening of (3), which carries a  $5 \times 7$  plate, etc., up to  $8 \times 10$ .

The camera, with arrangement for carrying the lantern slide plate, must be focused on the negative placed in the carrying kits. The support for the negative and the camera should be the same, and they should be firmly adjusted on supports of the proper height to bring the camera axis about to the middle of the negative. All the light which enters the camera *must* (not may or should)

come from the sky. I spoiled dozens if not hundreds of plates when I began making lantern slides, because I did not so illuminate my negatives. I followed the best books I could get; they all said a ground glass should be used. If camera and negative are horizontal, half the light comes from the earth and one-half the field will be less brightly illuminated than the other, ground glass or no ground glass. One day when there was snow on the ground, I made a fine lot of slides. I invited my neighbor Kodakers to see them; they wanted to see me "do it again." Of course I agreed. The snow had melted, and every slide was a failure. The next day I tilted my machinery as shown in the figure, until all the light was skylight, and I have had no trouble since. The sun, of course, must not shine on the negative.

The lantern slide plates must be fresh. Do not buy them of dealers. Send direct to the manufacturers. I learned this also by dear experience. I know how to use just one kind of plates and one developer. They give perfectly satisfactory results. I have no doubt that other plates and other developers would do as well, but they could not do better. Accordingly, without disparaging any brand of either plates or developers, I shall give exact details of how I make slides and what I use. Any beginner or any one else who cannot make slides that show in the high lights perfectly clear glass, can learn to do so if he will heed the following directions accurately: get Seed's lantern slide plates from the factory; use for a developer equal parts of "A" and "B" made up as follows:

A	{	Water	-	-	-	-	-	-	16 oz.
		Hydrochinone	-	-	-	-	-	-	120 grains
		Sodium Sulphite crystals	-	-	-	-	-	-	1 oz.
B	{	Water	-	-	-	-	-	-	16 oz.
		Caustic Soda	-	-	-	-	-	-	60 grains
		Potassium Bromide	-	-	-	-	-	-	60 grains

Arrange an apparatus like the one in the cut; point it at ten o'clock on a bright sunny day toward the southwestern sky. Five seconds exposure with a 128 stop and a medium dense negative would be the right time for my lens, an anastigmat, series III A. It will serve for a beginning time perhaps for any lens. If the time is not right it can be regulated; if the image begins to come between forty and sixty seconds development, a good slide can be made by arresting the development at the proper time, that is, when it is slightly darker than it should be, as it will wash out some in the fixative. If the image is clearly seen before twenty seconds, the exposure was too long; if only after ninety seconds, it was too short. These directions should be unnecessary, however, as no one should waste his time trying to make a lantern slide who cannot make a good negative; the cheapest way to learn how to make a good negative is to take a few lessons of a good landscape photographer.

If one has not to reduce his negative, a lantern slide can be made by contact exactly as a velox print is made; place the film of the lantern slide plate next to the film of the negative, and expose it to a perfectly steady source of light like an incandescent electric lamp or a Wellsbach burner; a coal oil lamp or a candle will do if the flame is always kept the same; two seconds, a yard away from a Wellsbach burner with a medium dense negative, will be a good time to try.

The density of a lantern slide should depend somewhat on the kind of light to be used in the projection. If this is to be acetylene or oil, the slide should be less dense than if the lime light or the electric arc light is to be used. Practice only can give accurate information in this respect, however. The beginner should practice with a negative from which a fine print has been made; he should have samples of the best work others have done with which to compare his own. Such samples can be had of any dealer in lantern slides. The landscape slides made by Wilson of Aberdeen, and for sale by T. H. McAllister & Co., 49 Nassau street, New York, are good.

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### The Technique of Biological Projection and Anesthesia of Animals.

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#### IV. SOLAR PROJECTION APPARATUS AND ITS ADJUSTMENTS.—Continued.

Having set the porte-lumière in its place in the window, darkened the room and arranged the screen, the microscope is adjusted as follows: Turn the handle

FIG. 3.—*Hydra fusca*. Example of low power projection, using 1-inch objective with porte lumière. Distance to screen, 30 feet.

(h) until the long axis of the mirror (m) points toward the sun. Rotate the mirror screw (s) until the beam of sunlight is thrown into the condenser (c) and is

seen to come to a focus squarely in front of the center of the condenser. A piece of paper held for a moment in the focus proves the necessity of the water tank, or heat screen (w), which should be filled with perfectly clear water. Rotate the mirror and adjust its slant so as to keep the focus of the light in the optical axis of the condenser and tank, and set the microscope in its place. Too much emphasis can not be laid on the rule that objectives of different powers must be set at different distances from the condenser. This distance is most readily measured from that face of the condenser nearer the stage to the plane of the surface of the stage. The following measurements were made on a porte-lumière fitted with a single condenser of four and a half inches diameter and six inches focal length combined with a sub-stage condenser having a focal length of thirteen-sixteenths of an inch. They may be used as a guide in determining the proper adjustment of a microscope to condensers of different focal lengths by applying the rule that the distances are increased when a condenser of longer focal length is used, and decreased when using condensers of shorter focal length. The distance from the surface of the condenser nearer to the water tank (w) to the surface of the stage was as follows, with 1 in. Wales objective  $8\frac{1}{8}$  inches, No. 3 (or  $\frac{3}{4}$  in.) Leitz  $7\frac{7}{8}$  inches, No. 6 (or  $\frac{1}{2}$  in.), Leitz  $6\frac{5}{8}$  inches, and B. & L.  $\frac{1}{2}$ -inch oil-immersion  $5\frac{1}{8}$  inches.

To find the position giving maximum and evenly distributed illumination over the entire field on the screen with any given combination of condensers and objective, proceed as follows: (1) Adjust the mirror so that the light will be reflected along the optical axis of the condenser and microscope. (2) Place a mounted microscopical object on the stage and bring a low power objective to an approximate focus upon it. (3) If the light on the screen is at first too faint to permit of accurate focusing, move the microscope nearer to the water tank and focus sharply. (4) The object may now be removed from the stage, provided the focus of the objective remains unchanged. Mark the position of the microscope and test the light when the microscope is moved nearer to the water tank or farther from it. If the field of illumination on the screen shows a blue color, the microscope is too near the water tank. If the light is faint, even at its best, the mirror may need adjusting, the condenser may not be set squarely in its cell so that the condenser and microscope are not in the same optical axis, or the focal length of the condenser may not be suited to the objective. When the best distance has been determined for each objective, make a permanent record for future reference. (5) A strong light, either pale blue, white, or slightly yellow, may be obtained by varying the distance of the microscope from the condenser. The yellow tint is to be chosen as it gives the best differentiation of transparent tissues. (6) A sheet of foolscap or other white paper should always be at hand ready to be used as a temporary screen between the condenser and water tank. One use for it is to cut off the heat from the objective whenever work is interrupted for a time, but its most important use is to so reduce the intensity of the light which is reflected laterally from the polished end of the objective that vision will not be blurred when looking at the objective in focusing down on an object. Opaque or semi-opaque objects mounted in balsam should be protected from the heat as much as possible, for they are much more likely to be damaged

than are objects mounted in water or in aqueous media. Years of experience with objectives in bright brass and nickel plated mounts, with the first case of damage from heat not yet noted, indicates that, if reasonable care is taken, no injury is to be feared from overheating. (7) A sub-stage condenser is recommended in all instruments intended for use with medium and high power objectives. A plano-convex lens of about three-fourths inch focus is the most satisfactory for use with the condensers described above. (8) If an ordinary microscope with inclination joint is used in the place of a regular projection microscope, great care must be taken in arranging the support so that the objective and body will be in the optical axis of the mirror and condenser. This rule becomes increasingly important when high-power objectives are used.

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## METHODS IN PLANT PHYSIOLOGY.

### I.

#### INTRODUCTORY.

The present series of articles aims to deal exclusively with the *technique* of laboratory work in Plant Physiology; they are not designed to be a complete outline of a course in physiological botany, nor a substitute for a laboratory manual. Many of the historical methods with which all botanists are familiar have been omitted except where improvements could be suggested. Although these methods have been worked out for use in a university laboratory, yet many of them on account of their simplicity or accuracy will be found suitable for application in secondary schools. The illustrations are from photographs taken by the author. Whatever value the methods may have is largely due to Professor F. C. Newcombe, of the University of Michigan, in whose laboratory they have been developed.

#### I. GROWTH.

1. **Dependence of Plants upon Oxygen for Growth.** This fact is best demonstrated by enclosing plants for several days in a chamber devoid of oxygen and making comparisons with controls. Select two salt-mouth bottles about 15 cm. high and prepare two strips of glass which will pass into the bottles and allow the stoppers to be inserted. Cover one side of the glass with white blotting paper, securing the end of the strip with a rubber band. Draw a heavy pencil line across each strip of paper at least 2 cm. from one end, then saturate them with water. Fasten two seedlings of peas (*Pisum sativum*) or lupine (*Lupinus albus*) upon each strip by means of rubber bands, bringing the root-tips upon the pencil line. Fill each bottle to a depth of .5 cm. with water which has been freed from air by boiling, and insert the seedlings. Close one bottle with a perforated stopper, allowing the seedlings to obtain atmospheric oxygen. Close the other bottle with a two-perforate rubber stopper; through one perforation pass a glass tube to the bottom of the bottle and connect it with a hydrogen generator. Pass a stream of *pure* hydrogen through the bottle for thirty minutes, then plug the open perforation in the stopper with a glass rod and fuse off the glass tube while

the hydrogen is running. (The fusion will be facilitated if the glass tube has been previously drawn out in one place to about half the standard diameter.) After twenty-four to forty-eight hours compare the amount of growth in the different seedlings.

2. **Minimum, Optimum and Maximum Temperature for Growth.** This important experiment requires but little in the way of apparatus for its successful performance if the preparations are carefully watched and the temperature not allowed to vary more than two degrees. Prepare seven culture jars or damp chambers, using for this purpose small glass jars (pint fruit cans answer well), having the inside lined with moist filter paper and the mouth closed with a cover of wood or cork, through which a thermometer is passed. Arrange the jars about the laboratory in places where the temperatures given below are found to be fairly constant. If there is a paraffin oven or incubator in the laboratory the higher temperatures can be obtained around and upon it; or construct a temporary shelf with one end near a register or radiator and arrange the jars along the shelf at distances which will give the desired temperatures. Make a small table for holding the seedlings in the following manner: cut a disk of sheet cork or thin lumber a little smaller than the mouth of the jar, insert three legs about 10 cm. long and bore five gimlet holes through the disk. Soak this table in water for three or four hours before using.

When ready to set up the experiment, place water to a depth of 1 cm. in the damp chambers, take corn (*Zea Mais*) or pea (*Pisum sativum*) seedlings of 3 to 5 cm. length and mark them with a drop of India ink applied with a fine brush 1 cm. from the tip of the root. Place the seedlings in the holes in the tables and place the tables in the damp chambers *immediately*. The chambers now need the continuous attention of one or two students in order to maintain constant temperatures (the work will be facilitated if the temperature of the room be kept constant). The chambers containing pea seedlings should be kept at temperatures of 8°, 20°, 22.5°, 25°, 30°, 35°, 40°C; those containing corn seedlings should be kept at 8°, 25°, 30°, 32.5°, 35°, 40°, 45°C. At the end of five to seven hours remove the five seedlings from each chamber and accurately measure the distance from the tip to the ink mark; average the measurements and record the amount of growth at the several temperatures; determine the minimum, optimum and maximum for each plant.

3. **To Determine the Distribution of Growth in Stems and Leaves.** Use young potted plants for this experiment and keep them under the most favorable conditions possible. Mark at intervals of 2 mm. the terminal three or four exposed internodes of the stem of a monocotyledon and also of a dicotyledon. The common Wandering Jew (*Tradescantia fluminensis*) is the most satisfactory monocotyledon for this experiment; the leaf-sheaths should be removed on one side to permit the marking of the stem throughout the full length of the internodes. The Periwinkle vine (*Vinca major*) is a very satisfactory dicotyledon for marking, as it grows well in the laboratory; only the shorter shoots should be used.

Mark also two or three immature leaves on each of these plants, covering the lower surface of the leaf with ink dots 2 mm. apart, both longitudinally and transversely. Make observations after three or four days to determine the place and amount of growth.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### VI. URIC ACID.

Uric acid is normally in urine in about  $\frac{1}{20}$  of 1 per cent. Abnormally it may be present in varying quantities in the form of bright yellowish, red colored crystals, rhombic in form and bunched together like a small pile of shingles. It may also be present in unusual quantity in the form of urates. The quantitative estimation of uric acid, free and as urates, is a little difficult, but should be done in all cases where it is suspected in abnormal quantities, or when a very careful examination of the urine is required. Proceed as follows: 250 c. c. of the 24 hours urine should be acidulated with acetic acid, boiled and filtered. To 200 c. c. of this filtered urine add 10 c. c. of hydrochloric acid, C. P.; set aside for 24 hours in a cool place. The urates will be decomposed into uric acid, which without the free uric acid will crystallize on the sides of the vessel. Decant the urine, scrape the crystals loose, add water and filter. Be careful to wash all of the crystals out of the vessel so that they may be caught on the filter paper. Before filtering the filter paper should be heated to dry it thoroughly and carefully weighed. After filtering dry the filter paper thoroughly with heat and weigh again. The difference in the weighs will represent the amount of uric acid in 200 c. c. of urine. In normal urine the difference in weight should be about 85 milligrams, which would be the amount of uric acid in 200 c. c. If 1200 c. c. of urine be passed the uric acid should be six times 85 milligrams or 510 milligrams or .510 grams.

A sensitive balance is necessary for the delicate weighing.

Uric acid is increased in the following diseases: Fevers, leukæmia, acute articular rheumatism, diseases of the spleen, whooping cough, chorea, etc. It is decreased in chronic diseases of the spinal cord, chlorosis, anæmia, gout with deposits, etc.

### CHLORIDES, PHOSPHATES AND SULPHATES.

For estimating the quantity of chlorides, phosphates and sulphates present in the urine, the centrifuge method is recommended. Either a reliable hand or electrical centrifuge may be used. The results are arrived at quickly and accurately.

*Chlorides.*—The percentage tubes are to be filled to the 10 c. c. mark with urine, add 15 drops of nitric acid to hold the phosphates in solution, add sufficient nitrate of silver solution (composed of 5 grams nitrate of silver dissolved in 40 c. c. of distilled water) to fill to the 15 c. c. mark. Close the tube with the thumb and thoroughly mix. Put in the centrifuge and rotate at the rate of 1000 revolutions per minute for three minutes. The normal range of chlorides by this test is 10 to 12 per cent. read directly from the tube. The divisions 1, 2, 3, etc., are 10, 20 and 30 per cent., being  $\frac{1}{10}$ ,  $\frac{2}{10}$  and  $\frac{3}{10}$  of the 10 c. c. of urine used for the test. Clinically it is only desirable to know whether the

chlorides are decidedly increased or diminished. They are diminished in most acute febrile diseases, diarrhoea, albuminuria, and in chronic diseases generally. They are increased later in most of the above diseases where at first they were diminished. A continued increase occurs in diabetes insipidis.

*Phosphates.*—Phosphates occur in the urine in solution and in the sediment.

In *acid urine* all the phosphates will be in solution.

In alkaline urine the phosphates of sodium and potassium will be in solution. The calcium and magnesium phosphates will be in the sediment.

In ammoniacal urine the same condition occurs as in alkaline urine with triple phosphate added to the sediment.

Test for phosphates as follows:

Fill a graduated centrifuge tube to the 10 c. c. mark with urine, add 5 c. c.



Hand centrifuge.

Graduated centrifuge tube.

Electric centrifuge.

of the magnesium mixture (ammonium chloride and magnesium sulphate, each 10 grams, distilled water 80 c. c., liquor ammonia 10 c. c.). Thoroughly mix and centrifuge for three minutes at a speed of 1000 revolutions per minute. The normal quantity is from 8 to 10 per cent. as read from the percentage tube. The phosphates are generally deficient in disease, occasionally they are increased. They are especially deficient in chronic Bright's disease, Addison's disease, pyuria, tuberculosis, carcinoma, etc. They are increased in epileptic attacks, small pox, cholera infantum, etc.

*Sulphates.*—The sulphates are usually in solution in the urine with the exception of calcium and magnesium sulphate, which when present may be found in the sediment. Proceed to test for sulphates as follows:

Fill a graduated centrifuge tube to the 10 c. c. mark with urine. Add 5 c. c. of barium solution (barium chloride 10 grams, hydrochloric acid 1 c. c., distilled

water 160 c. c.). Mix thoroughly and centrifuge it for three minutes at a speed of 1000 revolutions per minute. The percentage indicated on the tube will be 8 per cent., that is, a little below the mark 1 on the tube.

The sulphates are increased in febrile diseases, especially in acute rheumatism, pneumonia, diabetes mellitus and leukæmia. They are decreased in chronic diseases of the kidneys.

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## MICRO-CHEMICAL ANALYSIS.

### XX.

#### SILVER GROUP CONTINUED—LEAD.

This element is found in the 10th series of Group IV of the Periodic System. The other basigen in this group which we will consider is tin. Between tin and lead there is an, as yet, undiscovered element. On account of this serious gap it is simpler to discuss the micro-chemical reactions of lead in connection with silver than to attempt to do so with tin.

Lead can be satisfactorily detected in the presence of other elements by several reagents, the most important of these being :

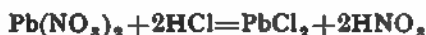
- I. Hydrochloric Acid.
- II. Sulphuric Acid.
- III. Potassium Iodide.
- IV. Ammonium Dichromate.
- V. Copper Acetate with Potassium Nitrite.

Of these reagents the analyst will generally select, for ordinary work, either III or V. But he will usually have already noted the effect of hydrochloric or sulphuric acids in either dissolving the substance or in applying a general group test.

The analyst must ever bear in mind the tendency on the part of lead to form basic salts and double salts. The former because of their insolubility often appearing as more or less amorphous precipitates and the latter frequently separating when least expected as beautifully crystallized compounds when other elements are being tested for.

In testing alloys practically the only solvent available is nitric acid. But when using nitric acid it must be remembered that lead nitrate is a salt of rather low solubility and that it is far less soluble in nitric acid than in water. If concentrated nitric acid has been employed for attacking the substance it follows that very little lead will have passed into solution. Lead nitrate crystallizes in beautiful clear cut transparent octahedra and the usual combinations of the isometric system. The worker in micro-chemical analysis should familiarize himself with the appearance and peculiarities of this salt in order to take advantage of its chance separation, which will enable him to remove the greater part of the lead which may be present and to obtain a pure salt upon which to work.

*I. Hydrochloric Acid added to solutions containing Lead causes the separation of Lead Chloride.*



**Method.**—Into a drop of a moderately concentrated solution of the substance to be tested, cause a drop of dilute hydrochloric acid to flow. This flowing in method is far preferable to the direct addition of the reagent to the test drop. In a few seconds, if the concentration is right, there will appear characteristic white, long, acicular crystals, crystallites in the form of Xs and more or less feathery masses (Fig. 79). By transmitted light the crystallites often appear to be black.

**Remarks.**—The appearance of the lead chloride separating varies with the concentration of the solution being tested and with the nature of the substances present. If the test drop is not sufficiently concentrated the lead chloride will not separate at once in the form of the characteristic crystallites, but will appear more slowly, prismatic forms being the rule. This question of concentration becomes a most important one if the substance contains salts with which lead chloride can unite to form double salts, as for example chlorides of the alkali metals and ammonium, for in such an event dilute solutions or even moderately concentrated ones fail to yield recognizable forms. Indeed it may be said that testing for lead with hydrochloric acid is not advisable in the presence of members of Groups I and II.



FIG. 79.

Lead chloride differs from the chlorides of silver and mercurous-mercury in being easily soluble in hot water, thus affording a simple method of separation. On cooling, the lead chloride no longer appears in the forms shown in Fig. 79, but assumes that of thin orthorhombic prisms, rhombs and hexagons (Fig. 80).

Recrystallized in the presence of chlorides of Group I, double chlorides result, which generally separate more slowly. The crystal form is quite different from that of the normal salt. It is quite important that the worker should be familiar with at least the double chloride of cesium and lead (cesium chlorplumbate),

since this compound not infrequently makes its appearance when testing for tin with cesium chloride and is quite apt to puzzle the beginner.

Alkalies convert lead chloride into a basic chloride to which the formula  $\text{PbCl}_2 \cdot 8\text{PbO} \cdot 4\text{H}_2\text{O}$  is generally assigned.

Thallous salts yield with hydrochloric acid star and cross-like crystallites differing considerably from those given by lead. There is little danger of confusing these two elements, since recrystallizing thallous chloride from hot



FIG. 80.

water, in which it like lead chloride is soluble, yields well formed cubes.

In the presence of chlorides of antimony and bismuth complex chlorides of low solubility are sometimes formed, against which the analyst should be on his guard.

*Exercises for Practice.*

To a drop of a concentrated solution of  $\text{Pb}(\text{NO}_3)_2$  add a drop of dilute hydrochloric acid in the manner described above. Make several other preparations varying the concentration of the test drops.

Recrystallize a preparation of  $\text{PbCl}_2$  by heating to boiling with a large drop of water.

Recrystallize a preparation of  $\text{PbCl}_2$  in the presence of  $\text{NaCl}$ , another in the presence of  $\text{KCl}$ , of  $\text{NH}_4\text{Cl}$ , of  $\text{CsCl}$ .

Test a solution of  $\text{Pb}$  and  $\text{Sb}$ . Then one of  $\text{Pb}$  and  $\text{Bi}$ . Then one containing all three elements.

To a preparation of  $\text{PbCl}_2$  add a drop of  $\text{NH}_4\text{OH}$ .

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## LABORATORY OUTLINES.

### For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

#### IX. *Slime Moulds (continued).*

##### (e) *Amœba* Sp. Class, Rhizopoda. Order, Amœbina.

If the student has not studied the *Amœba* in a general course in zoölogy, it should be taken up at this point, since the amœboid form probably represents the most primitive type of cell with which we have to deal. Amœbas can generally be found in the ooze at the bottom of ponds and creeks. To obtain Amœbas in large quantities, pack a glass jar rather tightly with *Ceratophyllum* or with pond lily leaves, and cover with water. The dish should be covered up. After a week or two, when the plants begin to decay, Amœbas will usually be abundant.

1. Scrape off some of the sediment from the *Ceratophyllum* leaflets and mount in water together with some of the brown scum present at this time in the jar. Under high power search for transparent, naked cells of irregular shape, which are slowly changing in outline by thrusting out pseudopodia. Sketch the outline of an individual six times successively, at intervals of ten seconds.

2. Describe the amœboid movement of the animal, and the formation of the pseudopodia.

3. Make a careful diagrammatic drawing of a large *Amœba*, showing the outer limiting layer (ectosarc), the inner more fluid granular part (endosarc), the nucleus (if distinguishable), the contractile vacuole, and the various ingested foreign bodies, as diatoms, desmids, etc.

4. NOTE—In the form following, a return will be made to a typical plant related to *Pleurococcus*.

X. *Scenadesmus quadricauda* (Turp.) Bréb. Order, Pleurococcales. Family, Pleurococcaceæ.

*Scenadesmus* is very widely distributed, and may be found in the sediment in the bottom of ponds, creeks, etc., along with diatoms and other microscopic plants. It usually consists of a colony of four, more or less spindle-shaped, green cells. The two outer cells have four slender, pointed, prong-like projections extending diagonally outward, one at each corner of the colony.

1. Mount some of the sediment containing *Scenadesmus*, and examine under high power. Draw and describe.

2. Compare a number of colonies as to size, shape of cells, and appearance of the projections.

#### ALGAE WITH FANTASTIC CELL WALLS OR WITH COMPLICATED CHROMATOPHORES.

XI. *Diatoms*. Order, Diatomales. Family, Diatomaceæ.

This family contains a large number of genera and species both living and fossil. Diatoms can always be found forming brown scums or sediments on the bottom of ponds, creeks, ditches, etc.

1. Mount some sediment or water containing diatoms and study the different species present.

2. Under high power, draw six different species, representing them from two to four inches long. They are unicellular plants with two silicified valves or shells which fit together like the lids of a pill-box. Represent carefully the markings on the shell. In some species the ends and central portion of the valves are marked by nodules and these points are connected by a rib or suture called the raphe. These can be seen from the valve view.

3. Notice the greenish, yellow or brown chromatophores, the nucleus, and the cytoplasm. How are the cell organs arranged?

4. Look for chains or filaments of diatoms, also for stalked forms.

5. Study dividing forms. Some species conjugate. Look for such forms.

6. Study the movement. Does it have any relation to the field of the microscope, or the intensity of the light in the field? Describe. What is the cause of the motion? Remember that the motion is magnified under the microscope. How long does it take a diatom to pass across the diameter of the field?

7. *Isthmia*. Scrape specimens of *Isthmia* from dry, red or brown algæ or study from mounted slides. *Isthmia* can usually be obtained from dry algæ collected on the California coast. Draw a specimen from the girdle view, showing the valves and details of the workings. Notice that the individuals are of very different sizes. Draw one showing the valve view. Draw an individual in process of division. Describe how the valves fit together, how new valves are formed, and what is the character of the valves of the two individuals resulting from a division. Explain the cause of the difference in size.

8. *Fossil diatoms*. Study material from the Tertiary deposit of Richmond,

Va. Place a fragment of the diatomaceous earth in a small bottle of HCl, crush gently and mount in water. Draw three different species.

9. *Note.* Diatoms, on account of the great number of forms, make a good study in variation. There is great variability without very much advance in structure or life cycle—horizontal evolution. Is there any special advantage in the great variety of fantastic markings on the valves? JOHN H. SCHAFFNER.

Ohio State University.

## A Review of the Existing Methods of Cultivating Anaërobic Bacteria.

### VII.

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New York State Veterinary College, Cornell University.

OTTO F. HUNZIKER.

**STEREO-MICRO-PHOTOGRAPHY.**—A small segment of the circle from the object-glass is stopped off by a black card, and a photo taken of a diatom or other objects. Next another impression is obtained on a separate plate, with an opposite and similar segment of the circle of the field stopped off. The resulting pair of pictures to be viewed with a stereoscope.

But as diatoms and scales of Lepidoptera are very thin and mounted flat, it is desirable in order to get a raised effect to enhance or exaggerate the perspective; therefore, for each exposure the object may be set a little out of the center away from the stop, and in addition slightly altering the illumination to get the best definition in both views. By these means the stereoscopic effect may be very much improved, and some remarkable results obtained in relation to the structure of insect scales and diatoms, which is still a question of uncertainty.—F. H. WENHAM, in *Eng. Mech.*

<p><b>SUBSCRIPTIONS :</b>          One Dollar per Year.          To foreign countries, \$1.25          per Year, in advance.</p> <p>Subscribers will be notified when subscription has expired. Unless renewal is promptly received the JOURNAL will be discontinued.</p>	<p>Journal of</p> <h1>Applied Microscopy and Laboratory Methods</h1> <p>Edited by L. B. ELLIOTT.</p>	<p><b>SEPARATES.</b></p> <p>One hundred separates of each original paper accepted are furnished the author, gratis. Separates are bound in special cover with title. A greater number can be had at cost of printing the extra copies desired.</p>
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The interest shown by science men everywhere in the American Association for the Advancement of Science foretells the success of the fifty-first annual meeting to be held at Pittsburg, Pa., June 28th to July 3d, 1902. Details of program, accommodations and railroad service have been carefully prearranged for the convenience and welfare of those in attendance.

The following general program has been announced :

**MONDAY, JUNE 30, A. M.**—Meeting of the council in Carnegie Museum room.

First general session of the Association at 10 A. M. in the Music Hall, Carnegie Museum.

Addresses of welcome.

Reply by President-elect Asaph Hall, U. S. N.

Announcements by the General, Permanent, and Local secretaries.

Agreement on hours of meeting.

Adjournment of general session, followed by organization of the sections in their respective halls.

**MONDAY, P. M.**—Addresses of vice-presidents, at three o'clock, before the following sections: Mathematics and Astronomy, Physics, Mechanical Science and Engineering, Geology and Geography, Zoölogy, Botany, Anthropology, and Social and Economic Science.

**TUESDAY EVENING.**—General session in the Music Hall, Carnegie Museum.

Address of retiring president, Charles Sedgwick Minot, of Harvard Medical College.

Reception to members and guests of the Association.

The succeeding days will be occupied with general and sectional meetings, the closing general session being held on Thursday evening.

The American Microscopical Society will meet in the Phipp's Botanical Hall, Pittsburg, Pa., on Friday and Saturday, June 27–28, regular sessions being held morning and evening of both days. The president's address will be delivered on the evening of the 27th. A very interesting and valuable program has been prepared, and it is hoped that all members will make a special effort to attend. At this time we again take occasion to call the attention of our readers to the desirability of all interested in the advancement of the microscope and of microscopical investigation becoming members of this, the only American society of microscopical workers of national scope. In union there is strength, and it is only through general coöperation of those who will be benefited by the improvements and advancements which are the aims of the society that results of importance can be attained. The editor will be glad to forward the applications of those of our readers wishing to be elected to membership at the coming meeting.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Karsten, G. Ueber die Entwicklung der weiblichen Blüthen bei einigen Juglandaceen. *Flora*, 90: 316-333, pls. 12, 1902.

The forms studied were *Juglans regia*, *J. cordiformis*, *J. nigra*, *Pterocarya fraxinifolia*, *Carya amara* and *C. tomentosa*.

In *Juglans cordiformis* at the time of fertilization and even later, the union of the carpels is delayed, thus leaving the ovules exposed so that the condition is hardly different from that in Gymnosperms with orthotropous ovules, e. g., in *Gnetum*.

There is an extensive sporogenous tissue in the nucellus, but any further development is usually restricted to a single cell in the median line.

The megaspore mother cell may develop directly into the embryo-sac or may give rise to a row of three or four potential megaspores, of which the two upper ones never develop, but the two lower seem to have equal chances. Many cases were found in which there were two embryo-sacs in the median line, one above the other, sometimes touching each other at the ends, but often separated by several layers of sterile tissue. A case is figured in which the sac next the chalaza has been fertilized. The egg and synergids of *Juglans nigra* cannot be distinguished definitely before fertilization. The union of the polar nuclei is delayed for a long time and perhaps does not occur at all. Double fertilization was observed, and the writer believes that, in all cases, the union of the male nucleus with the polar nucleus takes place earlier than the fertilization of the egg.

Although the occasional occurrence of hermaphrodite flowers may indicate a reduced rather than a primitive condition, the Juglandaceæ are to be regarded as one of the lowest families of the Angiosperms. Prof. Karsten would derive the Angiosperms from the Gymnosperms, making *Gnetum* the point of contact. His argument, based almost entirely upon embryological characters, is well summed up in his diagrammatic comparison of the *Gnetum* and Angiosperm embryo-sacs.

Gnetum embryo-sac	=	Angiosperm embryo-sac
Prothallium in lower part	=	Antipodals
Egg cells	=	Egg cell + synergids
Endosperm nuclei	=	Polar nuclei
The stimulus to development of embryo and endosperm by the fertilization of at least two egg cells.		} = { The stimulus to development of the embryo by fertilization of the egg and development of the endosperm by "vegetative fertilization."

C. J. C.

Strasburger, Ed. Einige Bemerkungen zu der Pollenbildung bei *Asclepias*. *Ber. d. deutsch. bot. Gesell.* 19: 450-461, pl. 24, 1901.

This paper emphasizes the fact that the embryo-sac mother cell and the pollen mother cell are homologous structures. There is the least deviation from the primitive condition when an

embryo-sac mother cell gives rise to four daughter cells, or potential macrospores, and the greatest deviation when the embryo-sac mother cell is transformed directly into a single macrospore, as in *Lilium* and other forms. The embryo-sac mother cell—and also the pollen mother cell—is at once recognized by the fact that at the first division of its nucleus the mitotic figure is heterotypic and the reduced number of chromosomes appears.

In the Cyperaceæ a pollen mother cell gives rise to a single pollen grain, but the mother cell in this case undergoes the customary divisions and one spore in its development resorbs the other three. *Asclepias* has been reported as a case in which a pollen mother cell gives rise to but a single microspore, just as the embryo-sac mother cell in *Lilium* gives rise to only one macrospore, but the present paper shows definitely that each pollen mother cell gives rise to a row of four microspores, each of which had been mistaken by former investigators for a mother cell. The heterotypic mitotic figure and the reduction in the number of chromosomes take place in the cell which gives rise to the row of four microspores, thus identifying it as the mother cell. As the matter stands now, there is no case of a pollen mother cell giving rise to a single microspore directly.

Since the protoplasm of the pollen mother cell of *Asclepias* is particularly free from granules, the writer made a thorough re-examination of the centrosome problem, using the latest methods of botanists and zoölogists, but was unable to find centrosomes. While the writer is not willing to assert that centrosomes will never be demonstrated in the higher plants, he is nevertheless of the opinion that they cannot be demonstrated by the methods now in vogue. C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

Deetjen. Untersuchungen ueber die Blutplättchen. Virchow's Arch. 164: 239-263, Taf. 1, 1901.

The author uses the following method to prevent the extremely thin blood-film from drying before fixatives can be

used: A drop of blood is spread on agar, not glass. The agar was prepared by taking a one per cent. agar solution in which, after filtration, .6 per cent. sodium chloride is dissolved; then .6 per cent. sodium metaphosphate and about .3 per cent. di-sodium phosphate ( $K_2HPO_4$ ). The metaphosphate alone preserves the life of the blood plates. A drop of this solution is allowed to flow over the slide and the agar cooled. Blood from the finger is spread on this cooled layer and covered with a cover-glass. If this preparation is examined on a warmed stage all the elements can be seen. The leucocytes move most actively in about five minutes; usually at the same time, often earlier or later, the blood plates show change of form. These structures put out amœboid processes and move with

great activity. This movement continues for as much as four hours after drawing the blood. Movement may last in the leucocytes for 24 hours after removal. The degree of activity depends on temperature. Movement may be seen at the ordinary room temperature, it is very active at blood heat, and more so at 40°C. Concentration of the salt is important. A greater per cent. impairs movement; weaker solutions to .4 per cent. are favorable, but lower cause swelling. Little or much di-sodium phosphate causes slower movement; too little  $\text{NaPO}_3$  causes a destruction of the plates, too much causes them to remain in a state of contraction. Fixation may be accomplished by the fumes of osmic acid or the direct application of osmic acid (1 per cent.) or Flemming's solution. The fluid is allowed to flow in from an edge and the process watched under the microscope for 3 to 5 minutes. Then the cover is lifted and stain used. Any anilin dye gives good results, but hæmatoxylin alone or with eosin is best for permanency. This process shows the blood plates to contain a nucleus that contains chromatin, sometimes in the form of a skein. This shows best after using Flemming's solution and methylen blue or Heidenhain's iron hæmatoxylin. This demonstration of nucleated blood plates, hitherto not obtainable, depends entirely on the duration of fixation. Over fixation prevents staining; cover-glass preparations as usually over fixed since good preservation of the hemaglobin requires more fixation than preservation of nuclei. Hence smear preparations heated for 2 hours at 120° are useless for blood plates. Preparations made as follows show these: fixation in 96 per cent. alcohol 1 to 2 minutes, air dried; then 5 per cent. formalin solution 3 to 5 minutes (this solution should be old and have stood in the light several weeks); washing in water without previous drying. Stain with Ehrlich's or Delafield's hæmatoxylin. The nuclei of the plates are now clearly blue; double staining with eosin shows a protoplasmic zone. Methylen blue and other anilin dyes stain deeply. In agar preparations the nuclei are still more apparent since the protoplasm is more expanded. No salt other than the metaphosphate gives these results; not ortho- nor pyrophosphate nor the salts of phosphoric and metaphosphoric acids. Some of the salts, as disodium phosphate, caused a precipitation of calcium salts so that  $\text{Na}_2\text{HPO}_4$  was first used and followed by 1 per cent.  $\text{NaPO}_3$ . This prevents the precipitation. The destruction of blood plates is not, however, thus prevented. The dependence of life-processes on salts of phosphoric acid is most clearly shown in that satisfactory agar is soon rendered unusable by boiling, and the plates quickly break up. Part of the metaphosphates become orthophosphates on boiling.

A. M. C.

Gurwitsch, A. Ein schnelles Verfahren des Eisenhematoxylinfarbung. Zeitschr. f. wiss. Mikros. u. f. mikros. Techn. 18: 291-292, 1902.

The author, after much use of Heidenhain's iron hematoxylin method, has devised a way to shorten the time of

procedure from 36 hours to 10 minutes. Many control tests demonstrated the accuracy of the results. Sections are fastened to the slide as usual, either with water or albumen, and paraffin removed. Sections are passed through the alcohols to water and then put in a large quantity of 2½ per cent. of iron mordant and set in the steam of an open water bath, where they are kept till the first bubbles rise, showing the beginning precipitation of the mordant. A rapid wash in water is followed by a similar procedure with the stain. On pouring the stain onto the sections in the steam they become black immediately. To obtain an intense stain it is desirable to keep the sections, with additions of stain, in the steam till the color has thickened on the edges and to add fresh color for a second time. Differentiation is accomplished at the ordinary temperature. There is neither alteration of tissue nor other injury to fear. The differentiation of difficult structures is as complete as with the longer time of staining.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoological Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Nickerson, W. S. On *Loxosoma davenporti* sp. nov. Journ. Morph. 17: 351-380, pls. 32, 33, 1901.

This interesting bryozoan, the first of the genus from American waters, occurs only in association with the annelid

*Clymene producta*. There are a large number of tentacles with unicellular suckers which serve for attachment in the absence of a foot gland. Ephemeral secretory structures, so-called "flask organs," are present, and in females with embryos an organ for nourishment is found on the floor of the atrium. The developing embryos are attached to this mammary organ and receive nutriment from it. Proterogynic hermaphroditism occurs, ova and later spermatozoa developing in the same gonad. There are no flame cells in the excretory organs, which consist of large vacuolated cells with ducts to the exterior.

No stupefying agent was found for *Loxosoma*, which is exceedingly sensitive. Material in extended condition was secured by the use of hot fixing agents such as hot  $\text{HgCl}_2$  or  $\text{HgCl}_2$ -formaldehyde mixture. Cytological material was fixed in Hermann's or Flemming's fluid. Iron hæmatoxylin gave the best results in staining.

C. A. K.

Ayers, H. and Jackson, C. M. Morphology of the Myxinoidea. I. Skeleton and Musculature. Journ. Morph. 17: 185-226, pls. 22, 23, 1901. I. Skeleton and Musculature (continued). Remarks on Homologies. Bull. Univ. of Cincinnati. Series II, 1: 1-15, pls. 1, 2, 1901.

These papers are based upon a study of *Bdellostoma* and the authors' conclusions lead to a revision of the generally accepted relationship of the so-called Cyclostomes to the vertebrate

phylum. The so-called tongue is in reality a transformed jaw apparatus, a detached lower jaw. The *Marsipobranchii* are thus true *Gnathostomes*, forming a primitive group which probably sprang from the common ancestry before the acquisition of paired appendages by the vertebrate type. The cranium in *Bdellostoma* retains its primitive condition, but the visceral skeleton is highly modified, the hyoid, first and second visceral arches are plainly represented and traces of others can be found. Material was killed and hardened in 10 per cent. formalin and preserved in a mixture of 95 per cent. alcohol, 6 parts; 2 per cent. formalin, 4 parts. Cartilaginous structures are well preserved, taking a pinkish tinge, affording excellent differentiation not present in alcoholic material. Non-cartilaginous connective tissue may take the same tinge.

C. A. K.

Buchs, G. Ueber den Ursprung des Kopfskeletes bei *Necturus*. Morph. Jahrb. 29: 582-613. Taf. 26-28, 1902.

Embryos were fixed in Zenker's fluid after Stöhr's method, and stained on the slide with borax-carmin and counter

stained with hæmalum and eosin. Sublimate-acetic (5 per cent.), followed by hæmalum and eosin, was also used. The author's results do not support the view advanced by Miss Platt, that the ectoderm shares in the origin of the visceral

cartilages. They are purely of mesodermal origin, and the ectoderm contributes merely to the nervous tissue.

C. A. K.

Richard, J. Les Campagnes Scientifiques de S. A. S. le Prince Albert I<sup>er</sup> de Monaco. 140 pp. avec 60 figs. dans le texte. Monaco, 1900.

since 1885, was prepared by Dr. Richard for the exhibit made by the Prince at

A most excellent *resumé* of the explorations of the ocean and its life, which the Prince of Monaco has carried on

the Paris Exposition. The work in part is historical, recounting the various voyages, and deals at length with the equipment for deep sea work and the most remarkable finds in the different zoölogical groups. Many excellent figures of deep sea animals occur in the text. The physical and biological equipment is quite fully described. Of especial interest is the self-spreading surface net, which makes it possible to sweep a strip seven meters in width. The net is so hung and so stretched by floats and weights that the wings spread whenever the net is drawn. The deep-sea traps devised by the Prince have yielded phenomenal catches even in the great depth of 5310 meters. As shown in the accompanying figure (A) a wooden frame supports a covering of netting into which large funnels lead from two faces. Smaller funnels (a) lead into smaller cylinders of wire mesh, designed for small animals such as *Amphipoda*. Bags of ballast (b) hold the trap in

Fig. A. 1. Trap in position; 2 and 3, details of joints and lacing; 4, float with cables and swivels.

place upon the sea bottom and a float of sufficient size carries the weight of the cable, which leads from the traps to the surface. It was customary to bait these traps with fish, refuse from the galley, etc., and the catch was usually composed of the more agile forms which escape the net and trawl. These traps have proved to be most useful additions to the equipment for deep sea work.

C. A. K.

Citron, Ernest. Beiträge zur Kenntniss des feineren Baues von Syncoryne Saraii. Arch. f. Naturgesch. Jahrg. 68 Bd. I., 1-27, Taf. 1, 2, 1902.

Material for this research was secured from a salt water aquarium which had been previously stocked with a few small colonies. Richly branched and exceptionally well developed colonies were found in this aquarium during spring months and produced swarms of medusæ. Young colonies were found upon the algæ, on shells of *Mytilus*, and on the sides of the aquarium. In this last position the growth of the hydrorhiza could be followed very readily. Concentrated solution of sublimate in sea water poured over the expanded animals was used as a fixing agent. Mayer's alum carmine or dilute gentian violet was used as stains. For the study of ganglion cells the hybrids were killed in one-half per cent. osmic acid, washed in water and placed in pyroligneous acid. After washing, these preparations were mounted in glycerine.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

*Zeitschrift fuer Allgemeine Physiologie.* Dr.  
Max Verworn. Bd. I, Heft 1. Jena, 1902.  
Verlag von Gustav Fischer.

This most recent addition to the already long list of biological journals makes in its first number an extremely

good impression. It appears at a fortunate time and can certainly lay claim to a field not covered by any previously existing periodical. At no time in the history of biological science has there been so widespread an interest in the problems of "general physiology" as at the present. Important investigations along this line are being prosecuted all over the world, and the number of investigators giving their attention to general physiology is constantly increasing. It seems eminently fitting then that this work should have a journal entirely devoted to its contributions, and this need the "*Zeitschrift für Allgemeine Physiologie*" seems destined to fill very satisfactorily.

The editor, Prof. Verworn, opens the number with an "Einleitung" of eighteen pages, in which he sums up the present status of the science of physiology, and sharply defines the lines on which the "*Zeitschrift*" is to be conducted. In this introduction Verworn answers in a very convincing way some of the criticisms which have been made against his own work, and admirably defends his standpoint. A practical point which will be noted with pleasure by Americans is that the "*Zeitschrift*" will publish contributions in any of the four ordinary languages of science: German, English, French and Italian. Besides accounts of original investigation a portion of each number is to be devoted to reviews of the current literature of general physiology, and also frequent "Sammelreferate" of the literature of special lines of work are promised.

The original papers in this first "Heft" are nearly all of a high order of excellence. The first of these is by Hans Winterstein, and is entitled, "Zur Kenntnis der Narkose." It gives the results of an investigation of the phenomena of narcosis studied by Verworn's method of substituting for the blood of the circulation, some other fluid whose effects on the organism, or the specific parts of the organism, it is desired to test. The most important result of Winterstein's work is the demonstration that in narcosis, not only the processes of dissimulation, but also those of assimilation, are paralyzed.

The second paper is in its general implications one of far reaching significance. It is entitled, "Neue Versuche zur Physiologie der Befruchtung," and is written by Dr. E. von Dungern. It is devoted in the main to a discussion of the causes of the specific nature of the process of fertilization. Why cannot the egg of the starfish be fertilized by the spermatozoa of the sea urchin? Briefly, the author finds the principal reason to be that the starfish egg contains substances which are extremely poisonous to the spermatozoa of sea urchins, but are

ineffective on the starfish spermatozoa. The normal serum of the rabbit was found to be antitoxic to this toxin of the egg. The chemical relations of these toxins of the eggs and other tissues of the body are very thoroughly worked out, and many interesting and important details are presented, of which space will not permit mention here.

The third paper is by Dr. Hans Friedenthal and is entitled, "Ueber die Reaktion des Blutserum der Wirbeltiere und die Reaktion der lebendigen Substanz in allgemeinen."

The fourth paper, "Inanitionserscheinungen der Zelle. Untersuchungen an Protozoen," by Hans Wallengren, is the longest in the number. The author describes very carefully and in great detail the changes which take place in *Paramecia* subjected to the process of starving. He found that after food supply had been withdrawn the organism lived at the expense of its own substance, using up first the endosarc, next the structures of the ectosarc, and finally the macronucleus.

Twenty pages of the number are devoted to reviews of current literature, a "Sammelreferat" under the title, "Alte und neue Vorstellungen über das Wesen der Nervenleitung," being contributed by Prof. H. Boruttau.

Altogether the "Zeitschrift" is to be most cordially welcomed, and wished "all success" in its career.

R. P.

**Loevenhart, A. S.** On the Relation of Lipase to Fat Metabolism—Lipogenesis. *Amer. Jour. Physiol.* 6: 331-350, 1902.

Kastle and Loevenhart demonstrated some time ago that the ferment lipase is reversible in its action, i. e., that it

is not only capable of splitting fat into a fatty acid and glycerine, but will also cause the recombination of these same two substances to form again fat. Loevenhart believes that in this reversible action of lipase is to be found the explanation of many hitherto very puzzling phenomena connected with the absorption, storing up, and utilization of fat in the organism. The enzyme is found to occur normally in all the organs which were tested for it, but most notably in the liver, active mammary gland, blood, lymph and intestinal mucosa. The author believes that the fat is taken up by cells of the intestinal mucosa in solution, is partially synthesized within the cell, but leaves it again to pass into the form of a solution. Fat is constantly being broken down and built up in the intestinal epithelial cell, the lipase establishing and constantly renewing an equilibrium of fat, fatty acid and glycerine. The fat appears to be carried in the blood and lymph in the form of free fatty acid, rather than as a soap. The storing up of fat in the tissues where it is found is brought about by the synthesizing action of lipase. The author's argument is well developed and supported in all points by experimental evidence; it seems to afford such a rational explanation of fat metabolism as has for a long time been greatly desired.

R. P.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, *Wesleyan University.*

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

**Macfayden and Roland.** The intracellular constituents of the typhoid bacillus. *Cent. f. Bac. u. Par.* I, 30: 754, 1901.

have devised a new method of study. The procedure adopted by Buchner of separating their constituents from yeast cells by grinding under pressure with sand, these authors reject on the ground that the method produces considerable heat and the heat will inevitably change the chemical nature of some of the delicate products. They, therefore, adopt a plan of destroying the bacteria cells and separating their contents which is free from this error. The bacteria are grown on the surface of agar and then washed from the surface, placed in a cylinder and rotated at a high speed so as to separate the organisms from the liquids. There is obtained a pasty mass which is then mixed with dry, clean sand and introduced into a metal cylinder provided with a jacket, through which cold water or brine can be circulated. A tightly fitted lid closes the cylinder, and through the whole a vertical axis is rotated provided with horizontal veins. The veins are revolved at a high speed, five thousand times per minute, while the cold brine circulates outside of the cylinder. The rapid rotation in a few moments results in the complete destruction of the bacteria, and it only remains to separate the sand and solid particles from the fluid to obtain a watery solution of the cell contents of the typhoid bacillus. This is done by pressing through silicious earth.

The authors further concluded that it would be advantageous if they could eliminate the sand from their method, and for this purpose devised a different method. The organisms were simply rotated in liquid air, the brittleness produced by the low temperature making it possible by the simple rotation to break the bacteria to pieces without the addition of sand. The process requires about two hours, and the material obtained contains the cell contents of the typhoid bacilli. The details of this method have not yet been given.

With the liquid thus expressed from the typhoid bacillus, the authors performed a number of experiments, and reached the general conclusion that there is no very active toxic material formed by the typhoid bacillus, growing upon ordinary laboratory culture media.

H. W. C.

**Holub.** Insecten als lebendes Substrat für Kultivierung ansteckender Krankheiten des Menschen und der Thiere. *Cent. f. Bac. u. Par.* I, 30: 284, 1901.

This author has devised a new and somewhat ingenious method of cultivating certain infectious bacteria. He worked chiefly with the organisms found

in ulcers. He cultivated the bacteria by inoculating the material of the ulcer into the body of insects. The inoculation was usually performed by simply in-

serting an infected needle into the body of the insect. In his experiments, which number many hundreds, he used species of all order of insects, and found that the bacteria would grow in insects from every order. The particular organism of the ulcers, developing in the insect, produces the death of the animal in three or four weeks. The body of the insect is then found to be filled with a practically pure culture of the organism. A long series of experiments has convinced the author that this method of using an insect as a culture medium is capable of development and of much practical use to bacteriologists. H. W. C.

**Griffon, V.** The Agglutination of Pneumococcus. Thèse de Paris, 1900-1901.

In order to investigate the serum reaction of pneumococcus we must have recourse to a special technique. One to 2 c. c. of serum should be inoculated with the pneumococcus and the culture should at once be put into an incubator at 37°C. for fifteen hours. When the human organism succumbs to an infection of pneumococcus, the serum scarcely becomes cloudy, and a microscopical examination reveals somewhat isolated diplococci which are distributed uniformly over the field, sometimes joined in little chains of from four to six members.

In the case of pneumococcus infection the agglutination may be macroscopic or microscopic.

1. When the agglutination is macroscopic or "pronounced," the serum remains clear and at the bottom of the tube may be seen a very distinct precipitate which presents different aspects. It may have the form of a peculiar cup-shaped buffy coating following the contour of the lower end of the tube; or it may consist of pseudo-membranous fragments which are multiple, flattened, or in ribbons; of irregular flakes; or of very fine dust-like grains which are held in suspension if the tube is shaken. These are the reactions which may be observed in the order of their decreasing intensity.

2. When the agglutination is microscopic or "slight," the inoculated serum will have lost its first clearness, and the cloudiness will be more or less marked. With the microscope can be seen either chains, or masses, or a mixture of both. From the edge of the masses chains may often be seen detaching themselves, rolling up, and forming around the islet from which they emanate a kind of collar, making it look like the head of a medusa. The chain is only the first step in the agglutination. The phenomenon takes place as soon as the microscope can be used and may become macroscopic after seven hours.

A drop of the culture on serum may be spread out, dried, and stained with a carbolated solution of methylen blue. In studying the pneumococcus serum reaction it is not necessary to have the serum strictly aseptic because the saprophytic germs of the skin do not develop rapidly enough to retard the reaction. The serum should not be coagulated. The pure culture of pneumococcus may be preserved in diluted blood or upon coagulated blood. Normal serum has no reaction. It sometimes happens, but only rarely, that the serum of a pneumonia patient will agglutinate only the pneumococcus of that same patient.

Tr. by Eleanor Larrabee Lattimore.

A. GIRAULD.

## NEWS AND NOTES.

**THE STAINING OF MAST CELLS.\***—Dr. Goldhorn has made some observations on the staining of mast cells and gives three methods by which these elements may be clearly demonstrated.

1. Saturate wood alcohol with dahlia or methylen blue and pour the solution on a freshly made blood smear without previous fixation. The preparation is then washed in water for a few seconds and dried in air.

2. The second method is one devised by the author, and considered by him the most simple in manipulation and universal in application to the various elements of normal and pathological blood of any stain yet offered to blood workers. With it erythrocytes stain pink, eosinophile granules more or less red, nuclei of leucocytes from blue to purple, and granules of mast cells most prominently metachromatic. The malaria parasite is stained as by Plehn's solution, but the nucleus of the young form is well seen. The nuclei of parasites in animal blood are brought out most beautifully. Blood platelets stain also.

The stain used in this method is prepared as follows: Methylen blue is rendered polychrome as directed on page 1635 of this JOURNAL and is then made strongly acid with glacial acetic acid. Next add 5 per cent. eosin solution till the mixture is transformed into a pulpy mass. Filter through two layers of filter paper and dry the mass left on the paper in a hot-air oven, after which it is dissolved in wood alcohol. If the solution when thus prepared is too acid, staining erythrocytes too deeply and leucocytes very little or not at all, the desired reaction may be obtained by rendering wood alcohol alkaline with potassium carbonate and adding it gradually until the dye gives the desired results. If the stain is made strictly neutral or alkaline, the red corpuscles will take on a greenish hue and the granules and anæmic degenerations will be shown while they will not be seen when the dye is acid. To use the stain it is only necessary to flood the slide for a few seconds and wash in water. Over-staining with a neutral stain is impossible. Eosinophile granules are seen by allowing the stain to act for about fifteen seconds. The strength of the stain may be altered by varying the amount of alcohol.

3. Another most satisfactory method is the following: Saturate wood alcohol with methylen blue and immerse the blood smear in this for about fifteen seconds. Wash in water and stain in .1 per cent. aqueous eosin for from fifteen to thirty seconds. If the smear is simply dipped into the eosin solution and the lower side of the slide wiped off so that a thin film of eosin is left on the upper side, the staining of the different granules may be observed under a medium power of the microscope; after the desired result is obtained wash and dry in air. The nuclei of leucocytes may be brought out more clearly if the smear is dipped in weak polychrome methylen blue before drying. To use the stain for mast

\*L. B. Goldhorn. Bull. Med. Sci. 2: 2.

cells some of the dye is dropped on the section and allowed to remain for thirty seconds. Differentiate in 95 per cent. alcohol until the pink color returns. With this method gonococci may be stained in smears. Dr. Goldhorn concludes from results obtained by the use of the above methods that the prevailing idea that mast cells do not exceed .50 per cent. in health is incorrect, the error being due to inadequate staining methods. His investigations have led him to the conclusion that the average percentage is over .75 per cent. C. W. J.

Our attention has been called to an error which appeared in the May number of the JOURNAL. About the middle of page 1775 the statement "The tree is monœcious" should read "The tree is diœcious."

### QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

24. Will some one give a simple method of preventing curling of paraffin sections during cutting by hand or microtome? A. H. D.

25. What is a satisfactory method of mounting simple microscopical objects such as fern or mushroom spores, etc.? B. B. B.

26. T. A. B. wishes to learn whether Eastman film cartridges answer the purpose for obtaining small photographs (4 x 5) in tropical regions. Would they be affected by temperature or the degree of moisture? Would they keep for a year or longer? If not, what is most desirable for that purpose?

### Books Received.

"The Microscope," An introduction to Microscopic Methods and to Histology. By Simon Henry Gage, Professor of Microscopy, Histology and Embryology in Cornell University, and the New York State Veterinary College. Eighth edition, cloth, \$1.50. Published by Comstock Publishing Company, Ithaca, N. Y.

"How to Improve Bad Negatives." By Edward W. Newcomb, Photo Expert, editor of Photo-American. Cloth, \$1.50. Published by the author, at Bible House, New York City. Postpaid, \$1.50.

"The Cow Pea" is the title of the latest publication issued by the Experiment Farm of the North Carolina State Horticultural Society at Southern Pines, N. C. This book, neatly bound and illustrated in plain and concise manner, discusses the value and uses of this important crop, the Cow Pea. Every reader can get a copy free by writing to the Superintendent of Experiment Farm, Southern Pines, N. C.

Bulletins University of the State of New York, 12, 13, 14, 15, 16, 17, 46, 49, 53, 55, 56, 57.

Summer Birds of Flathead Lake. Bull. Univ. Mont., 3:1.

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## The Hopkins Seaside Laboratory.

One of the earliest ideals cherished by the biological members of the faculty of the newly opened Leland Stanford Jr. University in 1890-'91 was the establishment of a marine biological station on the Pacific coast, in which might be utilized to the best degree its wonderful natural richness. The consideration of various points along the coast from the standpoints of faunal and floral resources,

### Hopkins Seaside Laboratory from the East.

accessibility and living accommodations was at once undertaken by Professors C. H. Gilbert and O. P. Jenkins, with the result that Pacific Grove, a small village on the southernmost headland of Monterey Bay, was selected as most suitable.

Upon the announcement of their plans the projectors met with most liberal co-operation from the citizens of Pacific Grove, the Pacific Improvement Company, and especially from Mr. Timothy Hopkins, to whose generous apprecia-

(1869)

tion of biological research the laboratory owes its existence to-day. As a slight recognition of his aid and sympathy the name of the Hopkins Seaside Laboratory was given to the institution. Upon a site donated by the Pacific Improvement Company the first building was erected and ready for occupancy by June, 1891, and to it was added the second building in 1894. The site is one of the most favored that could be selected there, being upon Point Aulon, a rocky head-

land jutting out into the bay, and at the same time but a short distance from the center of the town.

The bay itself is about twenty miles wide at its entrance, the broad sweep of sandy beach, broken here and there by rocky cliffs, extends almost uninterruptedly from Santa Cruz on the north around to Monterey on

#### Rocky Shores.

the south. At Monterey, two miles from Pacific Grove, the character of the shore changes, rugged granitic points jutting out into the bay at intervals, varied here and there by sheltered sand beaches or rocky coves. Here in the countless tide pools are the favorite shore collecting grounds. As the difference between highest and lowest tidal levels often reaches ten feet or more during the spring tides, a wealth of littoral life is rendered accessible.

In one of these coves, fifteen minutes walk along the shore from the labora-

#### Tide Pool.

tory, is located the picturesque and odoriferous Chinese fishing village which has proven the means of securing much of value from the waters of the bay. Though representing the lowest type of Cantonese, many of these fishermen can be turned into excellent collectors, if the financial consideration be large enough. However, their regular fishing is so profitable—especially during the salmon season—that it often requires an infinite deal of patience and perseverance to get anything from them at all. It was through the most intelligent of these fishermen, Ah Tuck Lee, that Dr. G. C. Price secured the first embryos of *Polisto-*

*trema* (*Bdellostoma*) *stoutii*, followed later by Dean, Ayers and Dofflein.

Beyond Point Aulon the same general character of shore continues, the cliffs becoming higher and more precipitous, with several rocky islets detached from them, the abode of countless gulls, cormorants and pelicans, who noisily contest possession with occasional seals and sea lions. Beyond Cypress Point, the restricted home of the weird *Cupressus macrocarpa*, another smaller bay opens, Carmelo Bay, at the mouth of the Carmel river. The rugged Point Lobos forms its southern boundary, beyond which the cliffs become sheer rock walls, rising abruptly from the ocean for hundreds of feet.

The two buildings of the laboratory are simple, unpretending structures, but admirably adapted for the purpose. The older one is sixty feet long by twenty feet wide. Its lower story is divided into two large laboratories, a store room, engine room and a dissecting room with concrete floor, suitable for work upon the larger marine animals. The second floor contains a general laboratory running the entire length of the building, and six private rooms for investigators. The second building contains a large, well-lighted basement with concrete floor at present used as a physiological laboratory. Above this the first story is divided into a large laboratory for advanced students, and six

Cormorants near Point Aulon.

private rooms. The upper floor has a large room fitted with blackboard and book shelves, used for lecture room and library, five private laboratories and a dark room for photography.

Each private room and laboratory is fitted with aquaria, small and large, and all the necessary glassware and reagents. An abundant supply of excellent microscopes, dissecting and compound, together with all needed physiological apparatus, is brought each summer from the University, and its library is drawn upon for the books and periodicals needed.

The salt water supply is pumped by a windmill into a 20,000 gallon tank, from which it is led into the two buildings and distributed to each room. The older building is piped with galvanized iron, the newer one with block tin, the stop cocks being of rubber. By this means a supply of perfectly pure sea water is assured. The fresh water supply is furnished by the excellent water system of the Pacific Improvement Company and is brought from the head waters of the Carmel river, twenty miles distant.

The usual supply of trawls, dredges and nets of various kinds and two boats are at the disposal of students and investigators. At present the laboratory does not own a steam launch, but for several years a gasoline one has been readily obtainable for dredging, and a new forty foot one is now owned in Pacific Grove, which may be chartered whenever occasion demands. The shore collecting,

however, has been of such surprising richness and has offered such a variety of problems that extensive dredging operations have not been necessary.

In the foundation of the Hopkins Seaside Laboratory the directors have had in mind three different but very closely related fields of usefulness, the same being filled so admirably on the Atlantic coast by the Marine Biological Laboratory under the able direction of Dr. C. O. Whitman. On a coast but scarcely

#### Chinatown Landing and Fishing Boats.

touched by the investigator of biological problems a most inviting field is offered, and it is the intention of the directors to give every opportunity in their power to men qualified for this work. To such the privileges of the laboratory are offered free of charge, and any assistance possible is gladly rendered.

The financial condition of the University during its first decade prevented the extension of any aid to the laboratory other than the loan of books, microscopes and other apparatus, so that the running expenses had to be met by students' fees for the most part. Hence it has been of course impossible to have the retinue of servants, collectors and fishermen, such as may be found in many

European stations where each table is endowed, often with a large sum. With but one exception the investigators who have availed themselves of the opportunities here offered have appreciated these conditions fully and have entered into the spirit of the laboratory completely.

The second aim of the laboratory is to supplement the work given during the remainder of the year in the regular courses

A Chinese Fishing Boat.

of instruction in zoölogy, botany and physiology at the University, by affording the students the opportunity of seaside study and to enable the more advanced to begin various lines of investigation under proper guidance.

A not less important field finally is the endeavor to raise the standard of scientific instruction in the public schools of the state by giving teachers and others facilities for becoming acquainted with the marine fauna and flora and the best methods of their study. For students receiving instruction, a fee of twenty-five dollars is charged for the session, to investigators the laboratory is free. A regular summer session of six weeks is held, beginning early in June.

The courses offered vary somewhat from year to year, those of the session of 1901 being the following :

I. General Zoölogy. Dr. G. C. Price. Lectures, laboratory dissections and field work upon representative forms of each of the larger groups of marine animals. In addition to the anatomical work the embryology of several forms is briefly followed.

II. Elementary Botany. Dr. G. J. Peirce. Lectures and laboratory work mainly upon the morphology and physiology of the marine and fresh water algæ, together with collecting and field study.

III. Advanced Course on the Structure and Physiology of the Algæ. Dr. G. J. Peirce. Character of the work to be determined largely by the previous training and inclinations of the student.

IV. Embryology. Dr. G. C. Price. Principally devoted to the development of the vertebrates.

V. Comparative Morphology and Histology of the Nervous System and Sense Organs. Dr. F. M. McFarland. Lectures and laboratory work upon a series of vertebrate and invertebrate forms.

VI. Advanced Invertebrate Zoölogy. Dr. F. M. McFarland. Detailed study of the morphology and classification of one or more groups of marine invertebrates.

VII. General Ornithology. Mr. J. Grinnell. Lectures on distribution, migration, moult, classification and economic relations of birds. Field study upon notes and habits, and laboratory work upon bird anatomy, plumage structures, identification of specimens and the preparation of study skins.

In addition to the above courses supervision of those beginning investigation in Embryology, Plant Physiology, Histology and Cytology was given by Drs. Price, Peirce and McFarland.

The following statistics of attendance during the first decade of the laboratory's existence shows a most encouraging condition :

Year.	1892	1893	1894	1895	1896	1897	1898	1899	1900	1901
Investigators and Instructors in private rooms . . .	6	6	8	7	11	7	15	16	11	12
Students taking regular courses . . . . .	13	25	46	43	19	46	54	52	48	40
Totals . . . . .	19	31	54	50	30	53	69	68	59	52

Though the regular instruction is limited to six weeks, the laboratory is open all summer and work may be continued independently without extra charges. Investigators desiring to use the laboratory during the winter months may readily make arrangements to that end with the directors, Drs. Jenkins and Gilbert.

Any attempt to give an adequate idea of the richness and variety of the fauna and flora of Monterey Bay would far exceed the limits of this article. Only the more striking forms may be touched upon here in passing. The most conspicuous of the marine algæ belong to the group of the *Phaeophyceæ*, the gigantic *Nereocystis* and *Macrocystis* forming floating beds along the coast, sheltering numerous animal forms. *Postelsia palmaeformis*, the sea palm, flourishes upon its rocks in the midst of the wildest surf, while acres of *Fucus* and *Lami-*

#### Chinatown and Boat Landing.

*naria* are exposed at low tide. The more delicate forms of red and green algæ are rarer and search for them must be made in more sheltered places.

The few classes of animal forms which have been studied in detail show a surprising wealth and variety. The lower Invertebrates, such as sponges, cœlenterates and worms, are especially abundant. Of the chitons twenty-five species have been recorded, among them the giant *Cryptochiton stelleri*, reaching a length of ten to thirteen inches, *Katharina tunicata*, several species of *Mopalia* and *Ischnochiton magdalenensis*, the development of which forms the subject of a recent very accurate study by Dr. Heath. Other forms of Mollusca are equally well represented; for example, some sixty species of Opisthobranchiata have been taken without any extended dredging. *Loligo* and *Octopus* are very common, the giant *Archoteuthis californica* somewhat rarer. The capture of squid for export to China for food and as a fertilizer upon the rice fields forms one of the principal industries of the Chinese fishing village, and after a favorable night tons of the animals may be seen spread out to dry in the sun. The squid are taken

during the dark of the moon, being attracted by pitch-pine fires built on iron cranes suspended over the water from a boat's side, while other boats encircle the inquisitive cephalopods with nets.

Echinoderms are abundant, among them ophiuroids, *Astrophyton* and at least seven species of holothurians. The occurrence of the hag fish has been noted

#### A Night's Catch of Squid.

#### Drying Squid.

already, and a Chimæra, *Hydrolagus collieri*, has furnished to Dr. Bashford Dean the first embryological material of this family.

The pelagic fauna is variable, but at times the surface is swarming with *Noctiluca* and other Protozoa, while Ctenophoræ, Pteropoda and long chains of *Salpa* are characteristic.

The zoölogical department of the University of California, under the energetic direction of Prof. W. E. Ritter, has for several years maintained a summer

#### Drying Squid.

#### Drying Fish.

biological laboratory, the site being shifted from year to year, at present, however, being located in more permanent quarters at San Pedro. To the northward Columbia University maintained for a short time a temporary laboratory on Puget Sound at Port Townsend, but other than this the Hopkins Seaside Laboratory and the San Pedro Laboratory stand alone in offering to the investigator and student facilities for attacking the many biological problems presented by the Pacific coast.

F. M. MCFARLAND.

Leland Stanford Jr. University.

### A Simple Device for Storing Fluid Culture Media.

In a laboratory in which certain fluid media are only occasionally used and in small quantities at the time, it becomes necessary to keep the medium in bulk. Then, every time the flask is opened and the cotton plug and neck of the flask flared, the neck either cracks or breaks, or the medium becomes contaminated if the neck is not flared sufficiently. Again, in a flask with only a cotton plug, the medium evaporates. To obviate these difficulties the writer contrived a simple device after considerable experimentation with several devices which had to be abandoned as impractical or inefficient. The device is readily understood from the accompanying drawing (Fig. 1). It consists of a flask, A, plugged with

cotton and sealed with a mixture of equal parts of paraffine and vaseline; a bent tube *d* and a syphon tube *a b c*. The flask containing the medium is plugged. The tube *a b c* passes through the plug, while the tube *d* reaches the upper layer of the plug, being only slightly embedded in it. The end of the tube *d* is loosely filled with cotton. The whole is sterilized, the plug pushed down the neck, leaving about one-half an inch space from the brim, which is dusted freely with powdered sulphate of copper and filled in to the point of overflowing with a mixture of sterile paraffine and vaseline. The object of adding vaseline to the paraffine is to render the latter softer and less retractive. This forms an air-tight stopper. The syphon is started by blowing through the tube *d*. Once started, the flask is inclined in the direction opposite

FIG. 1.—Device for Storing Fluid Media.

the outlet, when the fluid will run back into the tube *b*. The level of the fluid in *b* will be the same as in the flask A. The end of *c* is then sealed as well as the end of *d*. The sealing of the tube *d*, however, is unnecessary if the flask does not have to remain absolutely air-tight. To pour out the medium, the end of *c*, after careful flaming, is broken off at one end of the narrowed points, and the flask inclined in the direction of *c*. The rapidity of the flow can be made to vary from a drop to a stream, depending on the inclination of the flask. On this account, the idea suggested itself that this device could be utilized for dropping bottles. The regularity and certainty with which the fluid can be made to flow in drops render it equal if not superior to any dropping bottle on the market, while the cost is far less. Another utilization of this arrangement could be in the bacteriological examination of water when fractions of a c. c. must be used. The water may be poured directly into one of these sterile flasks, and the number of drops (at a certain rapidity of flow) per c. c. determined. It

is then an easy matter to take so many drops as are equal to a corresponding fraction of a c. c. By this method, the use of sterile pipettes is avoided, while the error is by no means greater than that occasioned by the use of several pipettes and dilutions.

A. ROBIN.

Delaware State Board of Health Laboratory.

## A System of Recording Cultures of Bacteria Genealogically for Laboratory Purposes.

The system here given furnishes a convenient means of recording all data relating to the study of individual laboratory cultures of bacteria. It was devised originally for stock cultures. As it served its purpose in that field very satisfactorily, it has been elaborated from time to time to meet further needs until it has reached its present form.

Consideration has been given to the system published by Pease<sup>1</sup> and used by him in his researches on cancer. In his system, both letters and figures are used to designate sub-cultures, the letters alternating with the figures, double letters, as AB, BC, being used when the alphabet is exhausted. In the writer's system, which is an adaptation of the Dewey Decimal system of classification,<sup>2</sup> figures alone are used, the method of use being such as is easily remembered.

The following is the writer's system :

Every species of bacteria, upon becoming a member of the laboratory stock, is given a number in the hundreds. Thus :

<i>B. coli communis</i> , . . .	100	<i>B. mallei</i> , . . .	400
<i>B. typhi abdominalis</i> , . .	200	<i>B. prodigiosus</i> , . .	500
<i>B. diphtheriæ</i> , . . .	300	<i>B. pestis bubonicæ</i> , .	600

*Individual specimens* of any one species coming from different sources, are numbered in the order of their isolation or reception with the units from 1 to 49. Thus :

<i>B. mallei</i> from one horse, . . .	401
<i>B. mallei</i> from a second horse, . . .	402
<i>B. mallei</i> from a different lesion in the second horse, . . .	403
<i>B. mallei</i> from same lesion at a different time, . . .	404
<i>B. mallei</i> from a third horse, . . .	405

The first culture of *B. mallei* isolated would be 401.1.

A sub- (or daughter<sup>3</sup>) culture from this original culture would be 401.11.

A sub- (or daughter) culture from this second culture would be 401.111, and

<sup>1</sup> Transac. A. P. H. A., Minneapolis, 1899.

<sup>2</sup> Decimal Classification and Relative Index. M. Dewey.

<sup>3</sup> For the sake of convenience and clearness, the following terms have been adopted in this article :

*Mother culture*.—The culture from which another culture is inoculated.

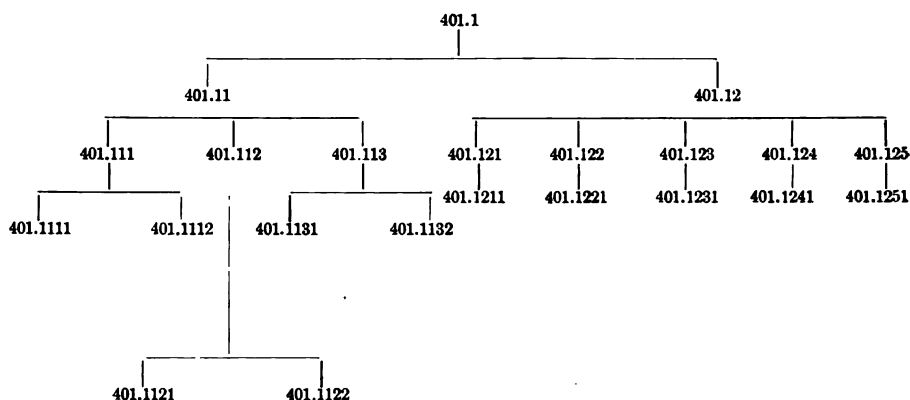
*Daughter culture*.—The sub-culture from the mother culture.

*Sister cultures*.—Two or more cultures made from the same mother culture.

(Obviously the terms are relative. It is evident that any one culture may be a mother, a daughter, and a sister culture at the same time.)

so on, each *sub-culture* bearing the number of the mother culture from which it was taken, with one figure more placed one space more to the right of the decimal point. If but one sub-culture is made, this added figure is always one. If more than one sub-culture is made, the first of these sister cultures is designated (as above) by the number of the mother culture with one in the next right decimal place, the second by two in the same place of decimals, etc., i. e., 401.11, 401.12, 401.13, etc.

This may be better illustrated by a diagram :



It will be seen from the above that a single daughter culture is always expressed by the number of the mother culture with the figure 1 placed in the next right place of decimals, and that further cultures made from the same given mother culture are expressed by increasing this last new figure in arithmetical order. This is the key to the system.

If at any time more than nine sister cultures be made from any one culture, the figures above nine are enclosed in brackets to avoid confusion, e. g., 404.18, 404.19, 404.1(10), 404.1(11), etc.

In cases where the numbers have become somewhat unwieldy, they may often be abbreviated by using exponents, e. g., 401.1111121113=401.1<sup>5</sup>21<sup>8</sup>3.

When an *unidentified* organism is *isolated*, it is given the specific number in hundreds which designates the species which it most resembles, but with the tens and units figures running above 50; thus, a glanders-like organism would be numbered 451, etc., pending its further examination. If found to be glanders, it would be renumbered below 450, taking the number next above that of the glanders culture last isolated.

Unidentified organisms, having no striking resemblance to any species possessed by the laboratory, are classed by themselves under one species number (e. g., 10,000) until identified.

A card system, used in connection with the above system of numbering, offers a complete and at the same time handy record.

The following card forms<sup>4</sup> are given merely as an illustration of the way in which the card catalogue system may be adapted to meet laboratory needs. They are not offered as a complete system, perfect in detail.

<sup>4</sup> The cards have been partially filled out the better to show their uses.

<b>401</b>		
Species <i>B. mallei.</i>	Source <i>Glanders case No. 38 (1901)</i>	
Isolated <i>Board of Health Laboratory</i>	by <i>R.</i>	Date <i>X-21-01</i>
History  <p style="text-align: center;"><i>Nasal discharge, bay gelding.</i>  <i>Owner, J. W. Clarke &amp; Co.</i></p>		

CARD I—Front.

**CULTURE RECORD CARD.**—The front of this card (I) contains all available information relating to the organism up to the time of its isolation. On the back of the card is printed a form (given below) which is designed to receive information relating to cultures of the organism subsequent to isolation.

<b>401</b>			
Culture	Date	Media	Remarks
<i>401.1</i>	<sup>1901.</sup> <i>10-30</i>	<i>G.P.</i>	<i>See Animal Card No. 38.</i>
<i>401.11</i>	<i>11-7</i>	<i>P<sub>2</sub></i>	<i>From testicles—typical growth.</i>
<i>401.12</i>	“	<i>A<sub>13</sub></i>	“ “ “ “
<i>401.13</i>	“	<i>P<sub>2</sub></i>	“ <i>site inoculation</i> “ “
<i>401.14</i>	“	<i>P<sub>2</sub></i>	“ <i>liver—no growth.</i>
<i>401.15</i>	“	<i>P<sub>2</sub></i>	“ <i>heart</i> “ “
<i>401.16</i>	“	<i>P<sub>2</sub></i>	“ <i>spleen</i> “ “
<i>401.17</i>	“	<i>P<sub>2</sub></i>	“ <i>omentum</i> “ “
<i>See Supplementary Card.</i>			

CARD I—Back.

Upon this form, entry is made of all cultures of the organism at the time of inoculation. The first column is reserved for the number of the culture, the

second for the date of inoculation, the third indicates the medium<sup>5</sup> upon which the culture is grown, while the fourth is for short notes and references.

Animals are regarded as one form of culture medium. Cultures made from different organs of the same animal are regarded as sister cultures. The name of the organ from which each individual culture came is entered in the column under "Remarks."

In cases where only nine or ten inoculations of media are necessary, the first card will contain the entire history of the work done. An extension card, printed on both sides with the same form as that given on the back of card I, may be used when more than this number of cultures is to be recorded.

<i>401.11</i>
<i>XI-10-01.—A thin, slimy, transparent, amber growth.</i>
<i>XI-15-01.—Color now dark brown.</i> <i>Growth opaque.</i>

CARD II.

CARD II. SUPPLEMENTARY CARD.—In any case where the results of an inoculation are too extensive to be satisfactorily stated on the culture record card, a blank card is used for this purpose. Such a card is identified by the number of the culture which it describes. A note referring to this card should be made in the "Remarks" column of the culture record card.

The culture record, extension, and supplementary cards are filed together.

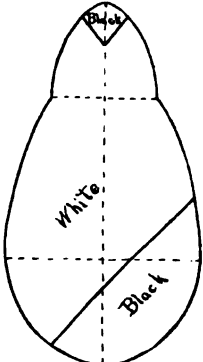
<sup>5</sup> A system of abbreviations and initials is used to indicate the media on which the culture is grown, thus:

G. gelatin.  
A. agar.  
M. milk.  
S. serum.

G. P. guinea pig.  
G. A. glycerine agar.  
P. potato.

A record is kept of the details of making of each lot of media, etc. (see Media card).

Each lot of each kind of media receives a number at the time it is made. These numbers, with the abbreviations above given (e. g., GA 13; A 12; etc.), serve to positively identify each tube of media.

Animal <i>G. Pig.</i>	Spec. rec'd <i>X-30-01.</i>	No. <i>38.</i>	Diag. <i>Pos.</i>
Sex <i>Male.</i> wt. <i>350 g.</i>	Bacterium <i>B. mallei.</i>		
Date inoc. <i>X-30-01.</i>	Material <i>Nasal discharge.</i>		
Site of Inoc. <i>Peritoneum.</i>	Source <i>Bay gelding.</i>		
	Owner, <i>J. W. Clarke &amp; Co.</i>		
	Symptoms <i>XI-5-01.</i>		
	<i>Testicles enlarged and adherent.</i>		
Died <i>Chloroformed XI-7-01.</i>			
Autopsy <i>XI-7-01.</i>			

CARD III—Front.

Autopsy <i>Both testicles adherent to scrotum.</i>
<i>Cheesy deposits scattered over surface of testicles.</i>
Mic. ex. <i>Preparations direct from testicles show typical glanders bacilli.</i>
Cult. <i>Cultures made from testicles on agar and potato. Cultures also made from liver, spleen, heart, omentum, and site of inoculation. (See culture record card.)</i>

CARD III—Back.

CARD III. ANIMAL CARD.—*Animal inoculations.*—In cases where animal inoculations are made, a special card is used in addition to those already described. This card is filled out as far as possible at the time of inoculation. A rude sketch of the color markings of the animal is made on the outline in the lower left hand corner of the card, which serves as a means of identification. The card is then hung, by means of a small clasp, directly upon the cage of the animal. It is thus conveniently at hand for the recording of symptoms, etc.

After the autopsy of an animal, the information given on the animal card may be transferred to the culture record or supplementary card. The animal card can then either be destroyed or filed separately.

<b>MEDIA.</b>	
Lot No. ....	Date .....
Weight of Meat ..... grams	
Amount of Water ..... c. c.	
Weight of Infusion ..... grams	
Weight of Infusion Used ..... grams	
Peptone ..... grams	..... grams
Gelatine ..... grams	..... grams
Agar ..... grams	..... grams
..... grams	..... grams

CARD IV—Front.

Titration.	Reaction		Amount of Reagent Used.		Remarks
	+	—	HCl	NaOH	
1					
2					
3					
4					
5					
6					
7					
8					
9					

CARD IV—Back.

CARD IV. MEDIA CARD.—The media card contains all information relating

to the making of the media, such as the amounts of the various ingredients, the titrations, etc. The media cards are filed by themselves.<sup>6</sup>

**LABEL WRITING.**—The culture number written on a culture tube is all that is necessary to completely identify that culture. Considerable time is thus saved in label writing. Other points, such as date of inoculation, media used, etc., may be added if desired, but they are not essential, since this culture number on the tube indicates directly: 1st—the species (by the number in the hundreds); 2d—the particular member of the species (by the number in the tens and units); 3d—the generation of the culture (by the number of places to the right of the decimal point); 4th—the culture from which it was inoculated (by the number as a whole, minus the figure in the last place of decimals); and this number also indicates indirectly (by reference to the culture card) all further information relating to the culture, i. e., the source, date of inoculation, the media, the lot of media, and in general all previous history.

**REMARKS.**—The card system has the usual advantages of cross references, convenience, and elasticity. By having a system of cards for media (including animals), and for cultures before and after isolation of the organism, one has a complete record conveniently at hand.

The criticism may be made that the system is complex. This is true, but it should be remembered that the facts which it aims to record are complex. In many cases where so complete a record as that provided for is not necessary, it can be considerably modified. On the other hand, it can easily be elaborated or extended to meet conditions not here specified. For instance, species might be designated by numbers in the thousands instead of in the hundreds (e. g., *B. coli* 1000, *B. typhi abdominalis* 2000, etc.), if a large number of different specimens of the same species are to be recorded.

The system as above outlined is now in use in the Bacteriological Laboratory of the Boston Board of Health. Modifications for the special purposes of other laboratories will probably suggest themselves. Those bacteriologists engaged in the differentiation and classification of species may find useful cards printed somewhat after the style of the Fuller and Johnson table<sup>7</sup> and designed to record the variation in reaction of different species or different specimens of the same species upon some one medium.

This card system is not offered in any sense as an alternative or substitute for the Fuller and Johnson method of tabulating final results of species work, but rather as an orderly and systematic method of accumulating the data from which such tables may finally be constructed.

BURT RANSOM RICKARDS.

Boston Board of Health Bacteriological Laboratory.

<sup>6</sup> The form here given is that used for artificial media, serum, agar, broth, etc., and is designed to conform to the methods of the Bacteriological Committee of the Am. Pub. Health Association.

<sup>7</sup> Transac. Am. Pub. Health Association, 1899.

## Fermentation Tube for Analysis of Gas Generated by Bacteria.

The following device has been found convenient for collecting gas in quantities large enough for an exhaustive analysis (Fig. 1). The fermentation-tube A is sterilized in live steam and filled with the medium while the stop-cock is open, the object being to fill the opening of the stop-cock with fluid. The stop-cock is then closed and the medium inoculated. At the end of 24 or 48 hours, when a sufficient quantity of gas has generated, the end *c* is filled with water and connected by means of a rubber tube with an eudiometer. By opening the stop-cock while gently blowing through the tube *a* the gas is expelled. The fermentation-tube can then be washed with sterile water and again filled with the medium plus the organism, and another quantity of gas obtained. This may be repeated several times until a sufficient amount of gas is secured. A convenient method

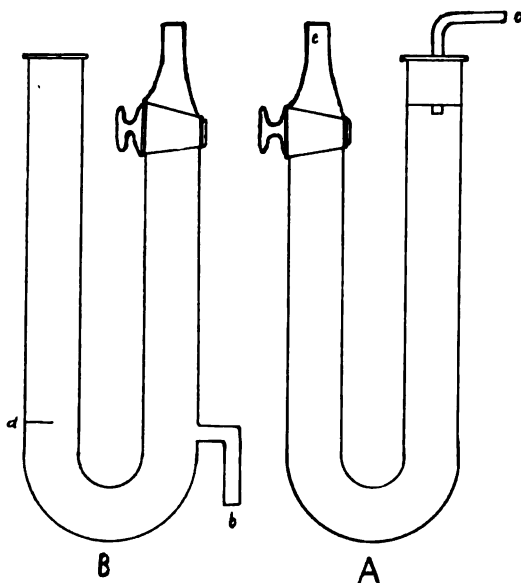


FIG. 1.—Fermentation Tubes for Collecting Gas for Analysis.

of analysis by absorption is to use the tube B. This is filled with the absorbing fluid, while the outlet *b* is held closed by the index finger. When filled to the mark *d* the open end is closed with a stopper, so as to prevent the fluid from running out through the side-tube. The latter is then connected with *c* and the gas expelled into B, while passing through the absorbing fluid. The non-absorbed gas may then be collected in similar manner for future analysis. The fermentation tube can also be utilized for making cultures from the growth in the closed arm. By means of a fine capillary pipette, fine enough to pass through the opening in the stop-cock, a sufficient amount of the culture may be obtained for further inoculation.

The tubes are manufactured by Arthur H. Thomas Co., Philadelphia, Pa.  
Delaware State Board of Health Laboratory.

A. ROBIN.

## Plant Preparations for Museums and General Demonstration Work.

Every teaching botanist recognizes the value of fresh plant material in the work of class instruction ; in most schools, however, fresh material is least available at the season of year when most needed, and in city schools is always more or less difficult to procure, so that the next best material in point of utility and economy is a useful supply of plant preparations. For some purposes it is even true that a skillfully mounted preparation is more valuable than fresh plant material which has not been carefully collected. That plant preparation is the best substitute for fresh plant material which best preserves the natural form and color of the object from which it was made. In the case of leaves and twigs

FIG. 1.—*Fucus vesiculosus*.

FIG. 2.—*Zea mays*. Stalks with adventitious roots.

good preparations are often made by carefully pressing and mounting the objects on heavy paper, but in the case of fleshy plants or organs different means must be used and the object is then usually mounted in some preserving fluid. Considerations of economy and utility require that the preparation be not removed from the medium in which it is preserved ; aside from the inconvenience, the necessary handling will inevitably tear and break up the material.

A good preparation should also have the element of beauty ; it may be useful and even valuable from a scientific standpoint without being beautiful. Not a little time and pains are often necessary in the selection of specimens in order to

secure handsome preparations, but it will be repaid by the improved appearance of the cases containing the preparations.

The preparations here described and figured belong to the botanical museum at the University of Michigan, and are used in various ways in class work in botany.

The method of manufacture is briefly as follows: The plant material is selected with a view to illustrating some particular fact or principle; it should be transported to the laboratory, allowing as little wilting as possible, especial care should also be taken not to bruise any part of the plant, as it often causes black spots. The material is thoroughly washed, using a soft brush if necessary to remove all adhering particles of earth, etc. After washing, the plants should be placed in a jar of two per cent. formalin for two or three days,

FIG. 3.—*Pleurotus petaloides*.

FIG. 4.—*Helianthus tuberosus*. Development of tubers.

in order to kill them and extract such of the coloring matter and juices as will come out readily; if this is not done the fluid in the permanent preparations often becomes cloudy.

Another method of killing which best preserves green colors, is by the use of a weak solution of copper salts. Several modifications of this method are in use in different museums: the following,<sup>1</sup> which is not original with the writer, has proven very satisfactory: The air is removed from the intercellular spaces of the plants by immersion in 96 per cent. alcohol or by immersing the plants in boiled water and placing them under the receiver of an air pump. When as much air as possible has been removed, the specimens are placed in a 5 per cent. glycerine

<sup>1</sup> Woods, Alfred F., Bot. Gaz. 24: p. 206.

solution containing 1 or 2 per cent. of formalin and enough copper acetate or copper sulphate to give the solution a strong greenish tint. This solution acts upon the chlorophyll to form copper phyllocyanate, which is insoluble in all preserving fluids except strong alcohol. At the end of two or three days the specimens will have assumed a dark blue-green color; they should then be removed and cleared in a dilute solution of glycerine and formalin which contains no copper salts, until the desired shade of green is reached, when they may be transferred immediately to the permanent preparation jars.

The most satisfactory style of museum jar is that shown in the accompanying figures; it is a rectangular, crystal glass jar with one polished side, the cover is ground and fitted to the jar. The usual sizes in which these jars can be obtained are 23 x 15 x 8 cm., 21 x 13 x 5.5 cm., 16 x 10 x 8 cm., and 12.5 x 9.5 x 6 cm. Such

FIG. 5.—*Cydonia Japonica*. Showing stipules.

FIG. 6.—*Dictyophora ravenelii*.

a jar is much better adapted to the use of plates for backgrounds than a cylindrical jar, besides obviating any contortion due to curved glass surfaces.

When a jar of the proper size has been selected, a suitable plate is cut for a background upon which to mount the specimen; this plate should be of such a height that it cannot rock backward and forward when the cover is on the jar, preferably it should be either of black or of milk glass, according to the color or transparency of the specimen to be placed upon it. The plate being laid in a shallow dish of water, the specimen is laid upon it and tied with silk thread of the same color as the background. The value and beauty of the preparation will depend largely upon the operator's skill in arranging the specimen on the plate; constant attention must be paid to the nature of the material, the facts to be illustrated, and the symmetrical arrangement of specimens. The jar is nearly filled with a 3 per cent. solution of formalin into which the plate bearing the specimen

is lowered so that the specimen is next the polished side of the jar; the final arrangement of the specimen is done after it is in the jar by means of a glass rod or stiff wire. To seal the jar it is only necessary to smear the edge of the cover with vaseline or with a mixture of bees-wax and tallow, press it firmly, and bind the edges. I have found lantern slide binders too narrow, and prefer strips of black paper 2 cm. wide attached with Lepage's liquid glue. The jars closed by this method never lose any of their fluid by evaporation or leakage, and if necessary they can be easily opened. No satisfactory directions can be given regarding labeling; if possible, the label should be on the front of the jar, if not, the label must be placed on the cover of the jar.

The value of such preparations must be apparent to all who have to do with teaching botany, or with making horticultural exhibits of any kind which are designed to be permanent. By means of the formol-glycerine-copper method of killing and clearing, all colors, including reds and yellows, may be preserved in

FIG. 7.—*Hydnum caput-ursi*.

their natural shades; pubescent, spiny, or fleshy material is kept without crushing or shrinking.

Many subjects capable of illustration by such preparations will suggest themselves to the minds of those familiar with botanical work. Figure 4 is one of a series illustrating the life-history of *Helianthus tuberosus*; Fig. 3 represents as fully as possible the life-history of *Pleurotus petaloides*. A collection of oak seedlings of different ages, showing the decreasing dependence upon the cotyledons, forms an interesting "Life-history series." Figures 2, 4, and 5 show what may be done by way of illustrating modified organs. Other preparations may be devoted to illustrating polymorphism, pathological organs, development of fruit from the flower, etc.

HOWARD S. REED.

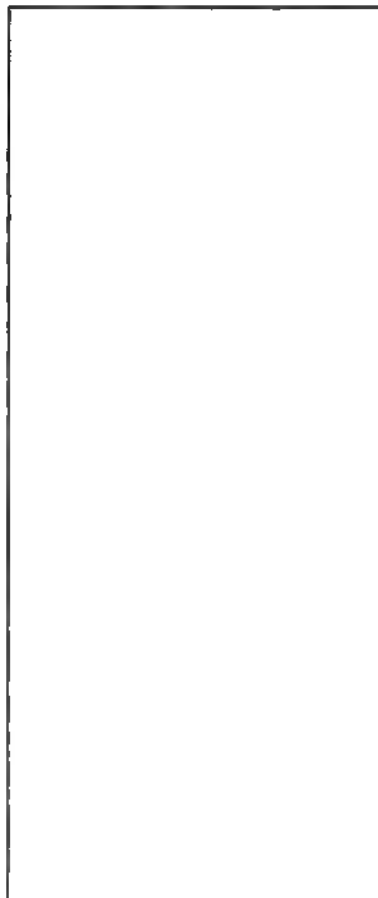
Botanical Laboratory, University of Michigan.

**LABORATORY PHOTOGRAPHY.**

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

**A SIMPLE VERTICAL PHOTOMICROGRAPHIC CAMERA.**

One of the chief factors in securing well defined images with the photomicrographic camera with moderately high power objectives, is perfect rigidity and stability, while in order to make it practical and workable it should be so arranged as to be adjustable both as to length of bellows and in relation to the microscope, and at the same time capable of being fixed rigidly in the desired position. To secure these ends, the construction shown in the accompanying engraving has been adopted. The base plate for supporting the microscope and the camera consists of a heavy iron casting with horizontal pillar into which is fitted a vertical steel rod one meter long. A thumb screw in the back of the supporting pillar bears against the rod and prevents its vibration, or rotation. A groove around one-half the circumference of the rod receives the end of the thumb screw and limits rotation to a half circumference. The camera bellows is attached to the vertical rod by means of two sleeves with thumb screws which bear against the vertical rod, holding the bellows in any desired position. Lateral motion is prevented by a longitudinal groove in the back of the rod which receives the thumb screws. It will thus be seen that a microscope placed upon the base plate can be brought to the center of the camera bellows when rotated to the limit of motion over the stage plate, and when the microscope is so centered, a locking gauge, shown at the front of the upper surface of the base plate, is brought up against the front of the microscope and clamped. The connection between the microscope and camera is made by a light-tight chamber, the front of the camera being dropped down by loosening the thumb-screw of the sleeve. The ground glass is then adjusted to the proper distance, when the apparatus is ready for exposure. When it is desired to adjust the object under the microscope the lower portion of the bellows is raised by loosening the thumb-screw and sliding the camera upward until the light-tight chamber is disengaged, when the thumb-screw on the supporting pillar is loosened and the camera may be swung to the left a half rotation out of the way of the microscope entirely, permitting the use of the instrument just as though it were not in connection with the camera at all. This makes the instrument particularly convenient for recording observations on living forms, and for general work.



A New Photomicrographic Apparatus.

L. B. E.

## METHODS IN PLANT PHYSIOLOGY.

## II.

## GROWTH—Continued.

4. **To Determine the Distribution of Growth in Roots.** Almost any seedlings having straight roots from 2 to 8 cm. long are suitable; they should be marked with India ink for a distance of 1 cm. at intervals of 1 mm., beginning at the tip. The ink may be applied with a fine brush or with a match which has been sharpened to a "feather point." Ganong<sup>1</sup> has proposed a very successful

method of marking roots by the use of a thread stretched by the spring of a piece of wire. If a drop of ink be placed on the thread and allowed to soak in, very even marks may be made with it without any danger of injury to the plant. The thread and wire may be mounted in a needle-holder. When the ink is dry, the root should be immersed in water for a moment and then suspended in a damp chamber.

The damp chamber is so important in all physiological work that it may not be out of place here to give the details of its construction. Select a glass jar suitable to the size and number of seedlings to be used, line the sides with filter paper, place water in the bottom of the jar to a depth of 1 or 2 cm. and allow the paper lining to become saturated; also line the lower side of the cover of the chamber with moist filter paper. For

FIG. 1.—A Damp Chamber. The bar is lifted out in order to show the manner of suspending the seedlings.

suspending the seedlings use a small wooden or glass bar about 1 cm. shorter than the diameter of the jar, cut two strips of white blotting paper the same width and length as the bar; moisten the strips with water and lay them on the bar, slipping a rubber band over one end. This end is now clamped in a vise and the strips of paper separated with one hand, while with the other a seedling is slipped in between them up next to the rubber band, the strips are then laid together and another rubber band is slipped on next the seedling. Continue inserting seedlings in this way until the bar is full (Fig. 1). Wedge the bar in the jar with a short piece of rubber tubing. Fastened in this way the seedling is uninjured, the root and stem are perfectly free to grow, and no difficulty arises from Sach's curvature. Within a few minutes after preparation the atmosphere of the damp chamber is saturated with moisture in which the seedlings will grow as long as the food supply lasts.

1. Ganong, W. F. Bot. Gaz. 27.

## II. RESPIRATION.

1. **To Demonstrate Respiration in Chlorophyll-bearing Plants.** Ordinarily the respiratory products are not demonstrable in the presence of those of photosynthesis; if the plant be deprived of light, however, the former are not obscured by the latter. The usual method of confining a plant under a bell-jar and excluding light produces good results if the temperature is kept fairly constant in order to avoid disturbances due to the expansion or contraction of air under the bell-jar. The edge of the bell-jar should be ground and it should stand either on a ground glass plate smeared with fat or in a shallow dish of mercury. After numerous trials it has been found that a potted *Coleus*, on account of its extensive leaf surface, gives better results than any other plant for this experiment. After twenty-four hours the presence of carbon-dioxid may be detected by a flame or by a saturated solution of barium hydroxid.

2. **To Determine the Composition of the Gas Evolved During Respiration.** The formation of carbon dioxid by respiring plants may be very successfully

FIG. 2.—An apparatus for determining the presence of carbon dioxid in the gas evolved by plants.

demonstrated in the laboratory or in the lecture room by conducting the gases from the culture chamber through a saturated solution of barium hydroxid and precipitating the carbon dioxid as barium carbonate. Set up the apparatus shown in Fig. 2. Bottle C contains three or four seedlings and a few pieces of moist blotting paper; the four wash bottles are filled with filtered barium hydroxid just before starting the experiment. Bottle F acts as an aspirator to draw a current of air through the apparatus. A clamp attached to the siphon is so regulated that the water flows in drops. At the beginning of the experiment bottle C should be connected directly with F and the aspiration current allowed to run until the air in the first three bottles has been replaced by air containing no carbon dioxid; when a liter or more of water has run off the bottles may be connected as shown in the figure. At the end of thirty minutes the solution in D should contain a white precipitate of barium carbonate, proving the escape of carbon dioxid from C. Bottles B and E are inserted to insure the removal of all the carbon dioxid.

University of Michigan.

HOWARD S. REED.

## The Technique of Biological Projection and Anesthesia of Animals.

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### V. PROJECTION MICROSCOPES USING ELECTRIC ARC OR OXYHYDROGEN LIGHT.

That the projection microscope is destined to fill an increasingly large and important place in class and lecture room work in colleges and secondary schools and in popular educational lectures, seems evident. Its general utility depends upon the success with which three practical problems are solved. First, a light of intense brilliancy must be produced and kept in the optical axis of the instrument. Second, the system of condensers must collect the largest possible percentage of light rays from the luminous point and deliver them at the proper angle of convergence for each of the objectives to be used. Third, the apparatus must not be too cumbersome or complicated or too expensive for ordinary

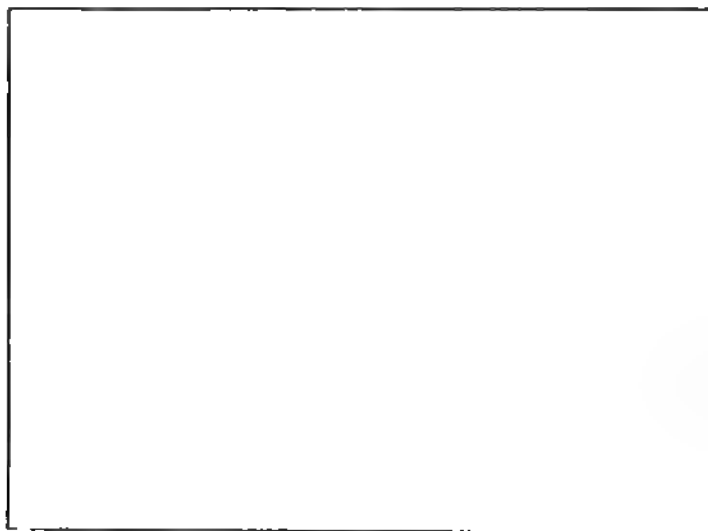


FIG. 4.—Projection Microscope with 90° Hand-feed Arc Lamp. The body is tilted up to expose the lamp and the electrical connections.

use. The instrument illustrated in this article is an attempt at a solution of these problems. The engraving shows the body rotated upward on the top of the plate, and held in position by a slender support so as to give a clear view of the 90° arc lamp and electrical connections.

The base board is cut away under the lamp so as to permit the use of long vertical carbons. At the rear end and right side of the board is placed the switch in the most convenient position for use. On the opposite side of the board is the fuse-block. At the right of the fuse-block, as seen in the illustration, are two binding-posts connected with the fuse-block by two twisted flexible wire

cables. From the other end of the fuse-block two similar cables connect with the binding-posts of the knife-switch. When the electricity is turned into the lamp by closing the switch, it passes to each carbon through the cables connected with the right hand binding-posts of the switch. The arc is formed between the proximate ends of the carbons which are shown as used on an alternating current of 110 volts, and in about the proper adjustment to develop the maximum power of the lamp. The carbons are fed together or singly by turning both feed-wheels at the end of the horizontal shaft below the horizontal carbon at the same time, or either one alone, as needed. The entire lamp may be elevated or lowered and rotated to the right or left, and moved along the base-rods and clamped in any position. The available light is derived from the end of the horizontal carbon and falls directly into condenser number one, then passes through condenser number two, both of which are in the condenser cell attached to the front of the plate. The light next passes through the water-tank, then through condenser number three, which is attached to the tank, then passes to the sub-stage condenser, if high power objectives are being used, next through the object mounted on the stage of the microscope, then through the objective and, lastly, through the amplifier and falls upon the screen. The sub-stage condenser is not used with low power objectives, as it produces a cone of rays having too wide an angle. The amplifier is a single plano-concave lens of five or six inches focal length and may be used with any objective to increase the magnification of the image on the screen. It is preferable to a regular microscopic ocular, as it intercepts less light.

A. H. COLE.

University of Chicago.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### VII—ABNORMAL CONSTITUENTS OF THE URINE.

Albumin in the urine in any quantity should excite the attention of the physician, and frequent tests should be made to determine its cause. The nitric acid test for the presence of albumin is very sensitive. Pour into a clean test tube  $\frac{1}{2}$ -inch of pure colorless nitric acid. Incline the tube and with a pipette allow to run down the side of the tube, very slowly, an equal or greater quantity of urine which will float upon the acid. This must be done carefully to prevent the least mixing. If albumin is present a white ring of coagulated albumin will appear at the point of contact. Colored rings are due to blood or bile pigments. A cloudiness higher up is not albumin, but is due to precipitation of urates.

It is much more desirable to test for quantity of albumin and quite as satisfactory and rapid.

Fill a graduated centrifuge tube with urine to the 10 c. c. mark, add 3 c. c. of potassium ferrocyanide solution (potassium ferrocyanide 10 grams, distilled water 100 c. c.) shake slightly and add 2 c. c. of acetic acid. Thoroughly mix

and centrifuge for three minutes or until the supernatant fluid is perfectly clear. The reading is made direct from the tube, each  $\frac{1}{10}$  c. c. of albumin in the tube meaning 1 per cent. bulk measured in the urine. When the percentage of albumin is high the urine should be diluted with an equal portion of water and the result multiplied by two. This method throws out and measures all the albumin and practically nothing else.

Albumin is found in the urine in acute diseases of the kidney, and in itself is not serious as it disappears upon recovery of the organ.

Chronic, persistent albuminuria when associated with epithelial casts in the urine means chronic Bright's disease. It is a serious symptom when persistent in the urine. If associated with high specific gravity 1024 to 1030, it is probably functional.

#### SUGAR.

Glycosuria may occur during the course of many acute disorders or result from various lesions, especially of the nervous system, but when pronounced and persistent it should be considered a case of diabetes mellitus, indicative of grave defects in the brain, liver or pancreas.

In testing for sugar in the urine, prepare Fehling's solution as follows:

##### Copper solution:

Copper sulphate, crystallized	-	-	-	34.64 grams.
Distilled water	-	-	-	500 c. c.
Mix and dissolve.				

##### Alkaline solution:

Tartrate of potassium and sodium	-	-	-	173 grams.
Potassium hydrate	-	-	-	125 grams.
Distilled water	-	-	-	500 c. c.
Mix and dissolve.				

The copper and alkaline solutions should be kept separately in bottles well stoppered with rubber corks.

Put one c. c. of the copper solution in a clean test tube,  
 one c. c. of water,  
 one c. c. of the alkaline solution,  
 one c. c. of water.

Shake and boil. The solution should remain clear. Add 1 c. c. of urine and boil gently. If the mixture turns a dirty greenish color as soon as it boils there is present about 1 per cent. of sugar. If the solution remains transparent throughout, or nearly so with no change except a change in the blue color, it may be considered free from sugar, but if it changes to a greenish hue after half an hour, about  $\frac{1}{10}$  per cent. of sugar is present.

The quantitative estimation of sugar is readily effected by means of Einhorn's saccharometer.

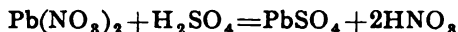
WILLIAM H. KNAP.

Harvey Medical College.

## MICRO-CHEMICAL ANALYSIS.

## XX.

## SILVER GROUP CONTINUED—LEAD.

II. *The addition of dilute Sulphuric Acid precipitates Lead Sulphate.*

*Method.*—To the rather dilute test drop add a drop of dilute sulphuric acid by the flowing in method. A dense granular precipitate is at once produced. This precipitate is generally at first amorphous, but soon becomes crystalline, or

to be more accurate, part of the tiny crystalline grains soon grow to a size and development permitting their easy recognition. Crystallites, lenticular grains, plates, and thin rhombs are seen (Fig. 81).



FIG. 81:

*Remarks.*—In solutions of substances containing other elements in larger amount than the lead, the precipitate of lead sulphate is usually amorphous, but when the lead is in excess, or in large amount, the precipitate is generally crystalline.

When the reagent drop is added at once to the test drop as in testing for calcium, strontium and barium, the precipitate of lead sulphate is apt to be amorphous.

Recrystallized from hot concentrated sulphuric acid in the manner employed in the cases of strontium and barium (q. v.) the crystals first obtained closely resemble those given by strontium, while a little later crystallites with feathery arms appear, which cannot be distinguished from those of the barium salt. This is to be expected since the sulphates of lead and barium are isomorphous. If after heating with concentrated sulphuric acid, the preparation is placed in a desiccator, thus excluding moisture from the air, the crystals separating have, according to Schultz, \* the formula  $\text{PbSO}_4 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ .

Lead sulphate can also be recrystallized by heating with strong hydrochloric acid or with strong nitric acid. In either case there is generally some decomposition and there appear on cooling, in addition to the rhombs of lead sulphate, crystals of lead chloride or lead nitrate.

Although it is seldom that the analyst will be deceived as to the character of an amorphous white precipitate obtained with sulphuric acid, or that he will meet with a precipitate consisting of the mixed sulphates of lead, barium, and strontium, he must be on his guard against such cases. When in doubt, advantage may be taken of the fact that lead sulphate is soluble in moderately concentrated solutions of sodium or potassium hydroxides, or concentrated solutions of ammonium acetate. In this manner a satisfactory separation may usually be effected. The decanted or filtered solution containing the lead can

\* Pogg. An. 133, 137.

then be acidified with nitric acid, evaporated to dryness, taken up with water acidified with nitric acid, and tested for lead with potassium iodide. Lead sulphate is also quite soluble in solutions of many other ammonium salts, a property it is well to bear in mind when engaged upon the analysis of complicated mixtures.

A convenient and simple method for ascertaining whether or not a white precipitate thrown down by sulphuric acid contains lead, is to draw off the mother liquor, then add to the sulphate residue a small drop of water and finally a crystal fragment of potassium iodide, after a few seconds the preparation is examined for the characteristic plates of lead iodide (Fig. 82).

#### *Exercises for Practice.*

Test a preparation of  $\text{Pb}(\text{NO}_3)_2$  with dilute  $\text{H}_2\text{SO}_4$ .

Recrystallize a preparation of  $\text{PbSO}_4$  by heating it with concentrated  $\text{H}_2\text{SO}_4$ .

Recrystallize another preparation by heating with strong  $\text{HCl}$ . Repeat the last experiment, substituting strong  $\text{HNO}_3$  for the  $\text{HCl}$ .

Make a mixture of  $\text{Pb}$  and  $\text{Ba}$ , precipitate with  $\text{H}_2\text{SO}_4$ ; then separate the  $\text{PbSO}_4$  by heating with  $\text{HCl}$ . Then try a mixture of  $\text{Sr}$  and  $\text{Pb}$ .

Try the method of separating  $\text{Pb}$  from  $\text{Ba}$  by means of  $\text{NaOH}$ .

Cornell University.

E. M. CHAMOT.

## LABORATORY OUTLINES.

### For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

XII. *Closterium* Sp. Class, Conjugatæ. Order, Desmidiæ. Family, Desmidiaceæ.

Desmids are quite common in ponds and lakes and species of *Closterium* can usually be found in the sediments at the bottom, on submerged water plants, or in large masses floating on the surface. Sometimes *Closterium* is very abundant in watering tanks, forming large, green, floating flakes.

1. Mount in water and observe the large bright green, unicellular plants which are more or less curved or crescent-shaped.

2. Draw an individual under high power, showing the cell wall with transverse striations in the central region, the two large chromatophores (chloroplasts) with highly refractive bodies (pyrenoids), the large nucleus with nucleolus in the central, clear space, and the peculiar vacuoles at each end. Notice the dancing, crystalline granules of calcium sulfate in the vacuoles (Brownian movement). Describe in detail, noting especially the symmetrical halves of the cell.

3. Notice the streaming of the cytoplasm between the large chloroplast and the cell wall. Trace the current around the end of the cell.

4. Look for dividing specimens. Draw and describe.

5. Search for conjugating individuals and for zygospores.

XIII. *Spirogyra* Sp. Class, Conjugatæ. Order, Mesocarpales. Family, Zygnemaceæ.

*Spirogyra* grows in stagnant water or slowly flowing streams, forming flocculent, floating masses of a bright green color which are slimy to the touch.

1. Study naked eye characters, noting that the mass is made up of slender free threads or filaments.

2. Mount a few filaments in water and examine under low power. Notice the cells with spiral chromatophores (chloroplasts). Shape of the filaments and cells? Count the number of cells across the cover-glass ( $\frac{3}{4}$  inch across). How many? Measure a long filament and estimate the number of cells it contains.

3. Draw part of a filament under low power showing ends, cells, and chromatophores. Any difference between the two ends? Describe.

4. Under high power, draw a cell showing the wall, spiral chloroplasts, pyrenoids, nucleus, and nucleolus. How is the nucleus connected with the other parts?

5. Draw a part of a chloroplast showing details of the margin and the pyrenoids.

6. Treat with salt solution. Draw and describe what takes place.

7. Study the conjugation from fresh material, or if this is not at hand, from material preserved in Petit's solution or from mounted slides. Notice two filaments side by side and that all the zygospores are in the cells of one filament, while the cells of the other filament are empty. This indicates a slight differentiation of sex individuals. Draw a piece showing a number of conjugated cells.

8. Draw two conjugated cells showing all details carefully, especially the zygospore and the conjugation tube.

9. Draw two cells in which the contents of one cell are passing through the tube.

10. Draw two cells in which the two rounded processes from the sides have just met.

11. Draw two cells in which the two processes are just beginning to develop.

12. Describe fully the process of conjugation as observed above.

13. Make a diagram showing the life cycle by means of diagrammatic figures of the plant, cells and spores.

14. Make a diagram showing the ancestors of one spore for five generations; take no account of vegetative propagation or of the possible close relationship of conjugating individuals (see Fig. 3). Compare with *Pleurococcus*.

Ohio State University.

JOHN H. SCHAFFNER.

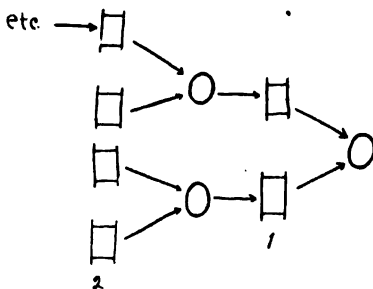


FIG. 3.—Life Cycle of *Spirogyra*.

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The annual report of the Director of the Office of Experiment Stations gives a glimpse of an organization of forces, gradually being perfected, which will undoubtedly be of inestimable value to agricultural pursuits and botanical investigation throughout the country. The agricultural experiment stations scattered over the United States have long been of great service to their immediate sections, but until recently there have been no efforts in the direction of coöperative operations under the guidance of competent authorities. Now the U. S. Department of Agriculture has undertaken to supervise the activities of this great number of isolated stations and endeavor by giving necessary assistance to economise money and time in researches which may come within the scope of the stations. Not only the stations of the states and territories on the continent, but also stations located in Hawaii and Porto Rico are to be included in this organization. At the head of the organization stands a body of men chosen from universities in every state in the Union and trained in special lines so that together they represent American botanical research. The society formed by these men—The Botanical Society of Washington—is perhaps second to none in the world as regards its personnel, and makes Washington the botanical center of America. With this body of professional botanists as the directing force and more than sixty outposts located in every section of the country, each under direct control of able science men, America must soon become one great experimental garden where each plot is made to serve the purpose for which it is best fitted and thus be of greatest benefit to the whole.

The work, however, to be successful, must receive the support of those not directly connected with its management.

The latest report of the Decimal Association issued from the London offices under date of June 4, 1902, states that there is a very decided growth of public opinion in favor of the compulsory adoption of the Metric System of weights and measures throughout the British Empire. The reform has many supporters throughout the colonies and the British consuls residing in countries where the Metric System is in use, urge the change from the present confused and complicated English System to the Metric System as one of greatest importance to British commerce. The recent favorable report by the Committee on Coinage, Weights and Measures in the United States Senate indicates that this country is ready to follow the lead, if not to lead, in the adoption of a system which has so much in its favor.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Lloyd, F. E. The Comparative Embryology of the Rubiaceæ. Memoirs of the Torrey Bot. Club. 8: 27-112. Pls. 5-15, 1902.

This paper is based upon a study of 28 species representing 9 genera of the Rubiaceæ. In general it may be said

that the archesporial tissue in the nucellus is quite extensive, consisting of from seven to fifteen cells, but according to the author, the pleuricellular archesporium is not a primitive character and has no phylogenetic significance. The megaspore mother cells divide twice, giving rise to a row of four potential megaspores, except when the development is arrested. Sometimes the nuclear divisions occur, but no walls separating the potential megaspores are formed. In the first division of the megaspore mother cell the spindle is multipolar and no centrosomes are to be found. In *Crucianella* and *Asperula*, where the cytological features were carefully studied, the reduced number of chromosomes is 10 and 12 respectively, but, after counting in a large number of cases, the writer believes that the number of chromosomes is not constant. In *Asperula*, in a late anaphase of the first division, the second longitudinal splitting of the chromosomes is already quite evident. In *Crucianella* this splitting takes place in the telophase. The divisions giving rise to a row of four potential megaspores, judged from a cytological standpoint, are true tetrad divisions.

The embryo-sac sometimes develops *in situ*, but sometimes passes out into the micropyle, moving like a pollen tube. In the *Galiceæ* the lowest of the three antipodals becomes very long and is plunged among the mass of disintegrating megaspores, which it absorbs. In *Diodia* a row of from four to ten antipodals serves the same purpose as the single long cell.

The suspensor development is quite remarkable. Lateral outgrowths penetrate the endosperm and act as haustoria, the nutritive function being even more highly developed than in the Leguminosæ.

C. J. C.

Frye, T. C. Development of the Pollen in some Asclepiadaceæ. Bot. Gaz. 32: 325-331, pl. 13, 1901.

This paper, which was already going to press when Strasburger's work on *Asclepias* appeared, is in perfect accord

with Strasburger's results as far as the most important question is concerned, viz., that in *Asclepias* the mother cell gives rise to four microspores as in other flowering plants. The mother cell is recognized by the reduction of chromosomes and the character of the mitotic figure. A most complete series was studied in *A. tuberosa*, but *A. Cornuti*, *A. phytolaccoides*, *A. incarnata*, *A. verticillata*, and also *Acerates viridiflora* and *A. longifolia* were studied sufficiently to warrant the conclusion that the development of the pollen is essentially the same in all.

The deeply staining granules described by Strasburger were observed by

the present writer, who describes them as dense fusiform bodies which appear about the time of the formation of the generative and tube nuclei. The fact that they persist even after the formation of the pollen tube, does not seem to agree with Strasburger's statement that microspores containing such bodies collapse later and become more or less completely crowded out. C. J. C.

**Korschinsky, S.** Heterogenesis und Evolution. Ein Beitrag zur Theorie der Entstehung der Arten. Flora, Ergänzungsband. 86: 240-363, 1901.

The publishers of *Flora* are to be commended for translating this important work on the origin of species and thus making it accessible to the

large circle of botanists who are not able to read Russian.

After a laborious and rather unprofitable study of plants in their native conditions, the writer turned his attention to garden forms. The most important result of his work is the conclusion that new forms do not arise through a gradual accumulation of individual variations, as Darwin believed, but rather that new varieties arise suddenly, showing at once a divergence from the pure species. This sudden appearance of new varieties the author designates under the term *Heterogenesis*. Not the least important part of the work is a carefully arranged summary of the literature bearing upon the origin of new forms in cultivated plants. C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Dekhuysen, M. C.** Ueber die Thrombocyten (Blutplättchen). Anat. Anz. 19: 529-540, 1901.

The author refers to Deetjen's work (see review p. 1859) and says he had already discovered the nucleated condition of blood plates.

He gives the following technic: *A.* Study of living cells. This is done in physiological salt solutions, rendered as nearly isotonic with the blood as possible. The osmotic pressure of the serum is determined by Bechmann's freezing apparatus (method given in Ostwald's physico-chemical tables). For amphibians and lampreys 8 per cent. salt solution is most favorable, having the same freezing point as the blood-serum. All glass vessels used in the preparation and preservation of this salt solution are carefully rinsed in fuming nitric acid and much heated; slides and covers, on account of the film of moisture on them, are similarly treated. Water is distilled in an apparatus made entirely of glass. Rubber is to be avoided. The salt used was purified by repeated melting and recrystallization. The normal salt solutions are preserved in syringe (syphon) flasks made entirely of glass. A glass tube with a stop-cock is fused to the longer tube from the bottle and the shorter is bent and filled with a plug

of cotton. (Such apparatus may be obtained from the firm of Franz Hegershoff, of Leipzig). A small high beaker is set on a Petri dish and covered with a larger one; all are cleaned and made very hot. Six such sets are prepared. The solution in the flask is heated to boiling and the short tube still kept closed, while the stop-cock is opened. The hot liquid is caught in the beakers, boiled for a short time and covered with the large beakers. The whole remains sterile for a long time, as in the case of bacteriological Petri dishes, and each glass contains from 40 to 50 c. c. of salt solution.

To study the living blood of a frog the animal is carefully wiped off, the skin cut through on the foot joint, and drawn back as far as possible on the lower leg and the joint is cut through with a scissors snip. The bleeding stump is washed off in sterile .8 per cent. salt solution and rinsed vigorously in the six prepared beakers of 8 per cent. solution. They are immediately covered. The liquid is allowed to settle and some of the sediment is taken up in a recently made, clean capillary glass tube. This is placed on the specially cleaned and sterilized slides and covered with similarly prepared covers. The author realizes that the process appears very laborious, but after once being done is not really so bad. The results are excellent.

*B. Fixation and staining.* The author uses 3-1 or 9-1 osmic-acetic (8 or 9 vols. of 2 per cent. osmic acid with 1 vol. of 6 per cent. acetic acid, containing  $\frac{1}{8}$  per cent. methylen blue.) A trace of acid fuchsin may be added. With invertebrates a trace of acetic acid sometimes produces an objectionable precipitate of granular albumen. Then osmic acid alone is used. Acetic acid has the objection that it decolorizes hemaglobin; with osmic acid this may with care be prevented. Not only must this decolorizing be prevented, but also certain fundamental changes of the stroma into a hyaline substance. Osmic-acetic (9-1) cooled on ice is peculiarly favorable to the demonstration of blood plates in the blood of man or mammals. The pricked finger or ear of a rabbit cut at the tip is vigorously stirred in the cold osmic-acetic.

A. M. C.

*Argutinsky, J.* Zur Kenntniss der Blutplättchen. *Anat. Anz.* 19: 552-554, 1901.

This author working on malarial parasites finds blood-plates to be nucleated bodies, as has been found by Deetjen, Dekhuyzen and Kopsch. He emphasizes the interesting researches of Nocht, who uses eosin-soda-methylen blue for differentiating malarial blood preparations. This stain was made by mixing 2-3 drops of a one per cent. eosin solution with 1-2 c. c. of water; to this is added drop by drop a solution made of one per cent. methylen blue with a half per cent. of soda; this liquid has stood for several days in a paraffin oven at 50°-60° and has been cooled. This addition is continued until the color becomes so dark that the eosin can hardly be seen. A preparation stained for 5-10 minutes, shows no precipitate and gives excellent nuclear stains. Such an alkaline stain quickly spoils, but that made naturally by the "alkaline methylen blue" lasts longer. The red stain thus made is called by Nocht the "red of methylen blue." Using the red derivative of methylen blue a very sharp chromatin stain is surely gained in malarial parasites and white blood cells. If a very thin malarial blood smear preparation, carefully fixed in sublimate alcohol, is stained

with a much diluted mixture of eosin-soda-methylen blue, the blood plates are clearly seen lying isolated or in very small heaps. Well-preserved plates are an intense red-violet, sharply outlined with a central part and an outer pale, clear blue border. The color of the central part resembles that of leucocyte nuclei. Many blood-plates in well-preserved preparations and most in poor ones are without sharply defined nuclei; but throughout the whole area of the outermost, pale, clear blue protoplasm are scattered intensely red-violet granules. These closely resemble the granular chromatin in many malarial parasites. The author considers all dry blood preparations, even those most carefully prepared, open to objection.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Houssay, Prof. F. La forme et la vie. Essai de la méthode mécanique en zoologie, pp. 924. Avec 782 figures dans le texte. Paris, 1900. Schleicher Frères editeurs. Prix 40 francs.

The method of presentation of the facts and theories concerning the structures, relationships, and functions of animals has become somewhat

stereotyped in text-books and manuals in recent years. Professor Houssay abandons largely the systematic basis and adopts what he is pleased to call a mechanical one, though in a broader sense than that usually conveyed by the term. The work is divided into three sections, "*Statique*," treating of the structure of animals from higher to lower groups, and the elements of structure, "*Cinématique*," treating of developmental processes, phenomena, and theories; "*Dynamique*," treating of the causes of the variations in forms discussed in the preceding section. The book is profusely illustrated, many new cuts from a wide range of original sources appearing in the text. Many diagrammatic figures of inferior quality illustrative of various theoretical phases of the subject add interest and variety to the work. Several of the chapters, notably those dealing with parasitism, larval forms, and polymorphism, are of more than usual interest by reason of their wide range of illustrative matter.

C. A. K.

Vaulleuard, A. Etude expérimentale et critique sur l'action des Helminthes. I. Cestodes et Nématodes. Bull. Soc. Linn. Normandie. 5e Ser. T. 4: 84-142, 1901. Review in Zool. Centbl. 8: 623-624, 1901.

Cestodes in the intestine of the dog and of fish do not produce in the lining of the intestine of the host at the point of their attachment any of the usual phenomena of irritation.

The cestodes produce no digestive ferment and thus depend upon food elaborated by their hosts. Their tissues contain poisons which on crushing are extracted by distilled water and certain other fluids. This extract if injected into the body-cavity of mammals or the frog causes death. The fluid is slightly opalescent and the poison is in part precipitated by alcohol and in part stays in solution. The dried precipitate is largely albuminoid, but a small

part going into solution in glycerine. The poison soluble in alcohol acts upon the musculature as curare while the others affect the central nervous system.

The amber-colored fluid pressed from *Ascaris lumbricoides* of the pig yields two poisons, one soluble in water but not in alcohol, and one in water and in alcohol but not in ether; the former affects the nervous system, the latter acts as curare. The author reviews the symptoms attending infection by cestodes and nematodes and finds among them many which may be referred to those produced by the injection of the poisonous extracts discovered by him. The pathogenic action of these parasites is thus chemical rather than merely mechanical.

C. A. K.

Girod, P. und Marshall, W. Tierstatten und Tiergesellschaften. 278 pp. Hermann Seemann Nachfolger. Leipzig, 1901. Mk. 3.00.

Professor Marshall's translation of this well-known treatise of Professor Girod upon the social organization of animals

is enhanced in value by his critical annotations which are extensive and serve to elaborate many points but partially developed by the author or present with greater precision the facts pertaining to animal societies. The association of various vertebrates, the social organizations of the invertebrates, the questions of commensalism, parasitism and colonial organisms fall within the scope of the work.

C. A. K.

Schaefer, F. Ueber die Schenckeldrüsen der Eidechsen. Arch. f. Naturgesch. Jahrg. 68: Bd. I, 27-64, Taf. 3, 4, 1902.

The skin was removed from the mesial face of leg from the cloaca to the knee and pinned out in the fixing fluid with

porcupine spines. Concentrated sublimate, picro-sublimate (concentrated aq. sol. sublimate 1 part, distilled water 2 parts, saturated aq. sol. picric acid 1 part), Fol's chrom-osmic-acetic mixture, and Müller's fluid were all used in the preparation of material. The concentrated sublimate caused some shrinkage of the tissues. Differential staining of the sections was accomplished with marked success with a combination of borax-carmin, Blochmann's modification of Van Gieson's method, and tetrabromfluorescin. Nuclei stain red, connective tissue blue, cornified tissue citron-yellow, and glandular tissue differently, according to the state of activity. Zander's methyl-eosin in 1 per cent. aqueous solution was used to demonstrate eleidin granules in material fixed in Müller's fluid. These granules stain a deep purple-red by this method, but are with difficulty stained after other fixing agents.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory, University of Michigan, Ann Arbor, Mich.

Veress, E. Beiträge zur Kenntniss der Topographie der Wärme-Empfindlichkeit. Arch. f. d. ges. Physiol. 89: 1-86. Taf. I-VI, 1902.

is of value both for its new results and on account of its contribution to the technique of the subject. The thermæsthesiometer used in the experiments is

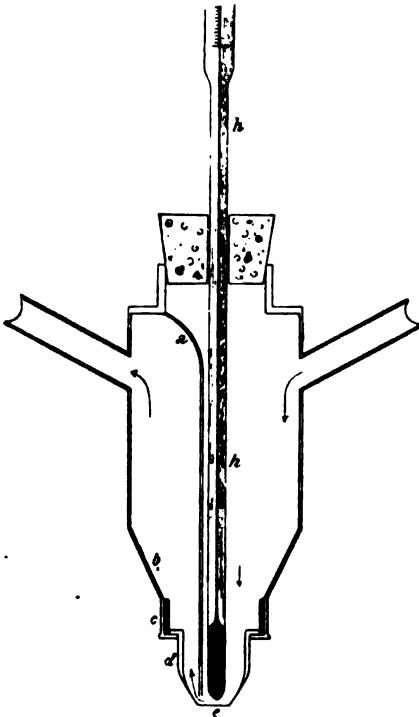


FIG. 1.

This very extensive and thorough study of the relative sensitivity of different regions of the human body for warmth is shown in section in Fig. 1. It consists of a metal cylinder 10 cm. long and 4 cm. in diameter, open above and below. Internally it is partially divided into two unequal parts by the metal plate *a*. From each of these two compartments a short tube extends, through which warm water may flow in and out respectively. The lower end of the cylinder is connected with a truncated cone, which ends finally in the end-piece *d* bearing the stimulating surface *c* which has a diameter of 6 mm. Through a cork in the upper end of the cylinder is passed thermometer *h* graduated to  $\frac{1}{10}$  Centigrade degrees. The bulb of this thermometer lies just over the plate *a*. The purpose of the partition *a* is to cause a constant flow of the hot water directly over *c*. When the apparatus was to be used its inlet tube was connected by rubber tubing with an "Hugerschoff's portable water heater," the water supply to which could be controlled by a screw cock,

and warm water was allowed to run through. The temperature in the thermæsthesiometer may be controlled by varying the rate of flow of water through the heater, and also by varying the height of the gas flame which furnishes the heat. When the thermometer registered 38-40° C. (approximately the temperature at which the sensation of warmth is first felt) the surface *c* was laid lightly on that portion of the surface of the body whose sensibility it was desired to test. The thermometer reading at the moment when the sensation of warmth was first felt was recorded, and then the temperature of the thermæsthesiometer was rapidly raised until this sensation gave place to a definite feeling of pain. The temper-

ature at which pain was felt was recorded. The author served as his own subject and tested the whole surface of the body. The results are graphically mapped on rather striking plates.

It was found that the two halves of the body are not equally sensitive to thermal stimuli, the left side possessing a greater average sensitivity. The regions bordering the median line of the body are less sensitive than the lateral surfaces, while the body as a whole is more sensitive than the extremities. The distal and the median regions of the extremities are more sensitive than the proximal and lateral. Fundamentally warmth sensibility depends solely on the abundance of the innervation; secondarily it depends on the relative thickness of the stratum corneum of the skin and on the effects of habit and use.

R. P.

Jordan, H. Die Physiologie der Locomotion bei *Aplysia limacina*. Inaug.-Dissertation, Bonn. (München: Druck von Oldenbourg) 1901. Pp. 51. Taf. I. (Also in Zeitschr. f. Biol. 41: 196.)

This work is a study of the physiology of the nervous system of *Aplysia limacina* with especial reference to the locomotor movements of the animal. A

series of extirpation experiments showed that parts of the body which are not in connection with the pedal ganglia are in a state of persistent tonic contraction. Removal of the cerebral ganglia, the pedal ganglion being left intact, causes persistent, rhythmical movements of the locomotor organs, which cannot be inhibited. The principal function of the cerebral ganglion under normal circumstances is to reduce the active condition of the pedal ganglion which tends to cause persistent movement of the organism.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt, Harvard University Medical School, Boston, Mass.

Walz, K. Leukämie. Centralblatt für allgemeine Pathologie u. pathologische Anatomie, 12: 967-1002, 1901.

The author presents a critical review of 223 articles upon leukæmia which have appeared since 1894. The section devoted to the ætiology of the disease is especially interesting. The rapid course which leukæmia runs certainly resembles that of an infectious disease.

The tonsillitis which frequently accompanies acute leukæmia or ulcers in the intestine, described first by Askanazy, might well serve as portals of entry for the infectious agent. Cabot (*Clinical Examination of the Blood*, New York, 1900) has reported a case of direct transmission of the disease from patient to nurse. Bacteriological studies and the earlier inoculation experiments yielded negative results.

By means of special staining methods Löwit has demonstrated peculiar bodies in the blood resembling protozoa which he regards as the cause of the disease. He further supports his claim by the artificial production in animals of a disease resembling leukæmia. As a specific stain he uses thionin followed by Lugol's

solution. Originally he used warm Löffler's methylene blue or an old thionin solution. In his latest paper (*Zeitschr. f. Heilk.*, vol. XXII, 1901) he recommends that the blood be spread with a needle, as the leucocytes are injured when too thin a film is made.

The method of staining is as follows: Fix by heating two hours at 120°C.; stain in concentrated aqueous thionin solution (Mühlheim) with the aid of heat; dry; counterstain in Orth's lithium carmine, which should be heated. Wash; dry and mount in balsam.

Löwit found in myelogenous leukæmia a large amœba within the lymphocytes (*Hæmamœba leucæmia magna*). In lymphatic leukæmia he found metachromatic stained bodies. They were present in only two cases in the peripheral blood, but more frequently in the blood forming organs. These also he regards as amœbæ (*Hæmamœba leucæmia parva sive vivax*). He describes with both hæmamœbæ various stages in the life-cycle—young parasites, crescents, sporulation and degeneration forms.

The views of Löwit have found a number of strong opponents, the most aggressive of whom is Türk. He asserts that Löwit's bodies are artefacts derived from mast-cell granules, and he was able to produce forms not distinguishable from Löwit's parasites in non-leukæmic blood. Vittadini (*Gazz. degli osped.*, 1900) is the only one who has fully confirmed Löwit's findings.

Pinkus (*Nothnagel's Spec. Path. u. Ther.*, vol. VIII, 1901) thinks it is questionable whether the bodies, even if they occurred exclusively in leukæmic blood, are the cause of the disease, since the *Hæmamœba magna* has been found in a case of lymphatic leukæmia, and, furthermore, in myelogenous leukæmia the parasites are not in the myelocytes, but in the lymphocytes, which are the least characteristic cells in this form of the disease.

Türk (*Wiener klin. Woch.*, No. 18, 1901) has never succeeded in transmitting the disease to animals. He states that the disease produced in rabbits by Löwit has nothing to do with myelogenous leukæmia, as the same disease-picture can be produced by inoculating portions of organs from individuals that have died of other diseases. Türk claims that the *Hæmamœba parva* is nothing more than the nucleolus of the lymphocyte.

Löwit in his exhaustive monograph (*Die Leukämie als Protozoeninfektion*, Weisbaden, 1900) answers the criticisms of his opponents and maintains the correctness of his observations. In his latest paper he describes the extra cellular forms of *Hæmamœba magna*.

J. H. P.

Loeb. Ueber Transplantation eines Sarcoms der Thyreoidea bei einer weissen Ratte. *Virchow's Arch.* 167: 175-191, 1902.

In an interesting series of experiments Loeb has succeeded many times in transplanting into other animals a Sar-

coma of the Thyroid. The original tumor was observed in a white rat. It was quite vascular, and in some places had undergone cystic formation. In all, 360 pieces were transplanted into about 150 animals. If the experiment was successful Loeb generally noted a growth ten to fifteen days later. Some rats were fed with particles of the tumor with negative results in each instance. In seven cases inoculations with the cystic fluid were employed and four of these gave

positive results. He observes that in transplanting portions of the tumor the greater part becomes necrotic. The morphological and physiological character of the tumor is generally preserved. Certain modifications of the form, however, occur under certain conditions, and it is possible to recognize some causes for these variations. Variations in the character of the cells may also occur, for tumors, which are typical spindle-celled sarcomata, may resemble on transplantation endotheliomata and appear on further transplantation like their original form. Tumors which have ceased to grow may increase in size after a portion is excised. Local causes, as possibly the pressure of the corrective tissue capsule, may hinder the tumor's growth. Secondary nodules are formed especially near an ulcerated portion of the tumor. Metastases may occur by contact, but no example was noted of metastases through the blood vessels or the lymphatics. Tumor particles already infected at the time of the transplantation may still cause a tumor formation.

W. R. S.

**MacCallum, W. G.** A Case of Multiple Myeloma. *The J. of Exp. Med., Balto.*, 6: 53-63, 1901.

The pathological features are here given of the case where clinical symptoms, including the occurrence of

albumosuria, had been already described by Dr. Hamburger in the Bulletin of the Johns Hopkins Hospital, 1901, xii, p. 38. At the autopsy tumor masses were found in the femur, ilium, clavicle, sternum and scapula, with pathological fractures. There was also a tumor mass projecting from the skull. These tumors were in general very soft and not sharply demarcated from the marrow of the bone from which they sprung. They were usually of a deep red color and showed everywhere a greyish tint. In nearly all of them definite nodules of firmer consistency and greyish-white in color were found. As in Wright's case, so here a delicate stroma was seen, in the rather wide meshes of which lay innumerable rather large round cells. The nucleus was large, round and vesicular, sometimes lying eccentrically. Two or three nuclei were at times found in one cell. Each nucleus was provided with a nucleolus and points and strands of chromatin arranged sometimes in a somewhat radial way. In dried smears, stained with Ehrlich's triple stain, the nucleus showed a certain similarity between its staining property and that of the myelocyte's nucleus. The protoplasm presented a rather ragged and granular appearance. With the triple stain it had a pale pink coloration and no specific granules were seen. With polychrome methylene blue of Unna or alkaline methylene blue it took on only the palest greenish-grey coloration. It showed nothing of the specific staining of plasma cells and exceeded them greatly in size. MacCallum comes to an opposite conclusion from Wright's and thinks these cells are derived from the large non-granular forerunners of the myelocytes.

W. R. S.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

Reed, Walter, and Carroll, James, of the U. S. Army. The Etiology of Yellow Fever. These investigators have already acquired an international reputation by the discovery that the mosquito is the means of distributing yellow fever. More recently, they have conducted investigations for the purpose of determining, if possible, the cause of this disease, and they presented at the last meeting of the American Bacteriologists an abstract of their results as follows:

They have ascertained that, in yellow fever, the blood serum of a patient, which has been filtered through a Berkefeld laboratory filter, is still capable of producing this disease when subcutaneously injected in small quantity (1 c. c.) into non-immune beings. They are able to report an attack of yellow fever after the usual period of incubation, in two out of three individuals thus treated, and to state further that the blood drawn from one of the cases produced by the injection of the filtered serum was capable of producing an attack in a third individual, when injected in small quantity; thus proving, as they believe, that the specific agent had really passed through the filter. They were also able to show that the blood in yellow fever, when heated to a temperature of 55° C for ten minutes, is quite innocuous if injected into susceptible individuals. The specific agent of yellow fever, therefore, is destroyed or markedly attenuated by this degree of heat.

H. W. C.

Saul. Beiträge zur Morphologie des Typhus Bacillus und des Bacterium coli commune. Berl. Klin. Woch. p. 1244, 1900.

The author has contributed a new method of the study of bacteria which he thinks likely to give additional data

for a proper differentiation of species. The method is based upon the well known fact that each species of bacterium produces a type of colony which is characteristic, but Saul insists that the characteristics are properly developed only after the colony has had a long time to grow. The new method devised consists of sowing the species in question in rather deep layers of agar and such a small number upon each plate that the colonies are widely separated from each other. The colonies are then allowed to develop for several months. After such long growth the agar is hardened by the use of formaldehyde, and sections made for study with the microscope. The author finds that closely allied species may produce colonies which are quite similar to each other when young, but after long culture of this sort develop distinctively characteristic colonies which are easily differentiated by the microscopical study of colony sections. In the present paper he describes and figures colonies of the *typhoid bacillus* and the *colon bacillus*, as well as of a *Staphylococcus*. The colonies appear quite identical in early stages, but when allowed to develop for several weeks and then studied by sections, they prove to be totally unlike each other and each characteristic of its own species. The author believes that this method of study will give a new method of differentiating closely allied bacteria. Certainly the figures given, which are taken from photographs, show these organisms whose colonies so much resemble each other as ordinarily studied, produce at the end of their growth quite different appearances. To what extent the method of study may be practical is a little doubtful, inasmuch as, at best, it requires many weeks before the differential characteristics of the colony are sufficiently developed to be studied.

H. W. C.

# Journal of Applied Microscopy and Laboratory Methods

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## A Modern Bacteriological Laboratory.

The purpose of this article is to present as clearly as possible a description of a modern bacteriological laboratory. I likewise wish to call special attention to those arrangements, by means of which a laboratory may contribute in the maximum degree toward the physical comfort of the worker, and also to the most economical use of his time.

The laboratory of the Department of Bacteriology and Comparative Pathology of Cornell University is doubtless one of the most recently equipped of the large laboratories in this country, and it is this one that I have taken as an illustration. A detailed description of it is given in order that those parts which experience has shown to be convenient, economical, and efficient may be applied by other institutions which are about to equip such a laboratory.

The reconstruction and reëquipping of this laboratory was rendered necessary by the disastrous fire in the New York State Veterinary College, in November, 1900. The laboratory, as it was originally arranged, was described in the JOURNAL OF APPLIED MICROSCOPY, February, 1898. At that time it occupied the west half of the top floor of the Veterinary building, while the east half was occupied by the laboratory of Microscopy, Histology, and Embryology. In the reconstruction, the upper floor was divided transversely, and the north half is now occupied by the Bacteriological laboratory, while the south half is retained for the laboratory of Microscopy, Histology, and Embryology.

The present equipment of the Bacteriological laboratory is the result of years of close observation and experience on the part of Dr. V. A. Moore, head of the department, who is a strong exponent of the practical, both in laboratory methods and in teaching. The laboratory is designed to accommodate one hundred and twenty-five students (twenty-five students at a time), each student working ten hours per week. To accommodate this number of students and supply them with a working equipment, so that each could do his work without delay or hindrance, was no small problem, but by the careful planning of Dr. Moore the problem has practically been solved.

The laboratory is supplied with twenty-five desks, each desk designed to accommodate five students, each occupying it at different periods during the week. The desk contains five drawers, one of which is assigned to each of the five students using the desk, as his own individual drawer. The desk is also supplied with three other compartments which are accessible to all the students working at that desk; one for the microscope, one for chemicals, and one for such articles as filter paper, clean cloths, glass rods, report blanks, etc. The desks are two feet wide, four feet long, two feet six inches high. They are made of oak, covered on top with a pad of heavy corrugated rubber, upon which rests a large plate of one-half inch glass, with rounded corners and ground edges,

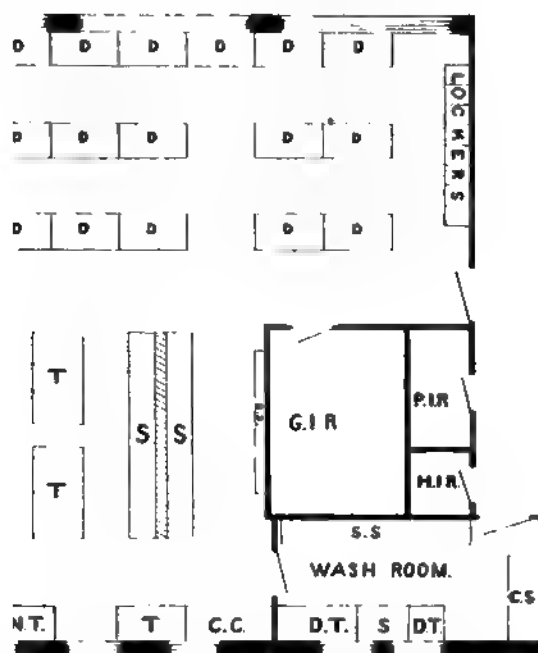


FIG. 1.—Plan of Laboratory. D-Desk, S-Sink, S.T Sterilizing Table, T-Table, N.T Nivellating Table, S.S Supply Shelf, B.S-Broad Shelf, G.I.R-General Incubator Room, P.I.R-Private Incubator Room, H.I.R-Histology Incubator Room, C.S-Cabinet for Supplies, C M-Media Cabinet, R.T-Reading Table, C.C-Chemical Cabinet, D.T-Draining Table.

covering the whole top of the desk. This provides a top which the student can keep thoroughly disinfected and spotlessly clean with a minimum amount of effort. A cut of this admirable desk accompanies this article. It will be seen that each student has the use of four drawers in the desk and a working space equal to the top of the desk, that is, eight square feet. Each desk is supplied with a reserve-flame Bunsen burner. The connection between this burner and the gas supply pipe is made by means of block tin tubing, and ground, gas-tight, brass unions, which permit the removal or exchange of burners if desired. This tubing, while it does not permit the moving of the burner as much as a rubber tube, is possessed of a certain amount of flexibility, and has certain advantages

over the rubber, in that it does not rot, does not permit escape of gas, and is safe in case the flame strikes back.

The desks are arranged in three rows facing the east side of the laboratory, which is lighted by eight large windows, reaching to the ceiling and occupying almost the entire side of the laboratory. These furnish a most admirable light for microscopical work. If the light is too intense during morning sessions, it may be softened by drawing the white shades with which the windows are provided. On dark days or afternoons during the winter months the incandescent light (16 c. p.), which is suspended over every desk, may be used. It is desir-

FIG. 2.—Individual Students' Desks and Lockers.

able in connection with these to use a screen having a metal base, formed of a small tin dish filled with lead, in which a wire is inserted bent at right angles at the top. Upon the horizontal arm of the wire is hung a sheet of common manila paper of such length that the lower part of it will be just above the stage of the microscope. As the student sits facing the light, the eye-shade is placed between the source of light and the microscope, excluding the light both from the eyes and from the upper surface of the object. By the use of this screen no ill effects on the eyes result from artificial illumination. By skillful manipulation of mirror and condenser excellent results may be attained in the way of lighting the field.

The heat and odor common to a bacteriological laboratory make the matter

of ventilation of prime importance as regards the comforts of its occupants. This is admirably accomplished by means of a system of ventilating fans with which the entire building is supplied. These force a continual stream of fresh air into the room. The automatic thermo-regulators maintain a constant temperature of 70°F. In addition to the physical comfort, there are at least three other essentials of a well equipped laboratory: (1) Facilities for keeping the apparatus clean. (2) Adequate facilities for making culture media and for sterilizing. (3) Adequate incubator space. To these a fourth could be added, namely, an arrangement by which the confusion in a laboratory, occasioned by students moving about in procuring their various supplies, is reduced to a minimum. These essentials have all been worked out in this laboratory.

There is absolutely no excuse for not keeping "things" clean. I have already suggested the ease with which the glass-topped tables are kept spotless. The floor of the laboratory is of Norway pine, and receives an occasional coat of oil, so that dust may be easily wiped up; windows are tightly fitted, so that no dust can blow in from the outside. Being located as it is on the top floor, annoyance from this source is small. Two large sinks, each twelve feet long and supplied with twenty faucets, ten of hot and ten of cold water, furnish ample facility for cleaning apparatus. Above each sink are hung six drying boards, containing 144 pegs each, inserted into the board at an angle of 30°, so that test tubes, bottles, etc., after being washed, may be placed on these pegs, allowing the dripping to fall into the sink. These two sinks occupy remarkably small space, being placed near the center of the room, back to back, with a partition extending up between them to cover the plumbing and support the drying boards. Perhaps a better idea of these sinks may be obtained from Fig. 3. A further aid to cleanliness is the fact that all work tables used in making media are covered with glass or sheet lead.

The question of furnishing sterilization facilities for so large a class was a difficult one. After some experience Dr. Moore was convinced that live steam, under low pressure, is the most successful method for sterilizing media. He had specially constructed eight sterilizers of various forms, all connected with live steam, and drain pipes leading from them. A temperature of 99.5° to 102°C. is reached in these sterilizers. It has been found that a large percentage of the media heated but once for twenty minutes is rendered sterile, thus practically making unnecessary fractional sterilization.\* Fitted within each of the sterilizers are specially constructed wire baskets, which afford a handy and safe way of putting in and removing media from the sterilizers. Large granite-ware pails and other utensils may be placed within these sterilizers, enabling one to make large quantities of media for stock. The use of live steam has a great advantage over nearly every other sterilizing process in that it is quick, safe, and economical. It is especially convenient for making media, as it does away with the slow water bath process, and at the same time eliminates the possibility of scorching your material. In addition to live steam sterilizers, the laboratory is equipped with a large sized Arnold steam sterilizer and a mammoth hot-air

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\* See article by Dr. R. C. Reed, in *Am. Mic. Journal*, 1897.

sterilizer. All these sterilizers are grouped together on a large stone table, fitted with gas, steam, and water. Over this table, and projecting a foot on either side, hangs a large hood into which passes the steam escaping from the sterilizers. In capacity these sterilizers are able to supply quick, convenient, and certain sterilization for a section of twenty-five students at one time.

Another excellent feature is the fire-proof incubator room containing the mammoth incubators. The fire which previously destroyed this laboratory was supposed to have been started from the incubators, and in the reconstruction great care was taken to prevent the possibility of a recurrence of the disaster.

FIG. 3.—Sink with Drying Boards; Sterilizing Table with Hood.

A room 10 by 12 feet, thoroughly fire-proof (asbestos walls lined with sheet tin, cement floor, etc.), is placed in the south end of the laboratory. It communicates by one heavy sliding door with the laboratory. In this room are placed the incubators for the use of students. Another room, smaller in size, but similarly protected, and contiguous to the former, contains the private incubators of Dr. Moore. In the room for the use of students are placed two large incubators, made specially for this laboratory. A cut of one of these incubators accompanies this article.

In large classes there is usually great confusion caused by students getting their cultures mixed, and difficulty in getting at cultures, especially when stored on a shelf in common with a number of others. Besides it is impossible to keep

the temperature constant when so many persons are using the incubator, owing to the continual opening and closing of the door. Dr. Moore has reduced this confusion, and the difficulty of maintaining constant temperature, to a minimum.

Each of these large incubators contains 48 drawers, made of galvanized iron, 8 inches deep by 5 inches wide, by 20 inches long, divided into two equal com-

FIG. 4.—Large Incubator for Students' Use.

partments, with sides and ends perforated with large holes to permit free circulation of air. The front of this drawer is of copper, and extends beyond the sides so as to form a continuous shoulder, and when inserted into the iron framework of the incubator fits tightly, thus reducing loss of heat when door is opened. A name plate holder and a handle by which to pull the drawer out is attached to the center of this copper plate. The iron framework containing the

drawers is enclosed in a cabinet fitted with two glass doors in front. By opening these doors the drawers may be removed or returned. One of these drawers is assigned to each student, and in it he is able to place all the cultures which it is necessary for him to have at any one time during his course. These incubators are heated by hot air supplied by a Bunsen burner, or two if necessary, which are placed in a chamber under the drawers. From this chamber a large number of brass pipes pass up along the back and two sides, conveying the heat to another chamber above the drawers, at the same time giving off heat to intermediate portions of the cabinet. From the upper chamber ventilation is secured by means of an adjustable ventilator. The temperature is governed by a Roux

FIG. 5 —Students' Lockers, Library, and Media Cabinet.

bimetallic regulator. It does not vary more than one degree in the different parts of the incubator, notwithstanding the fact that it is in constant use by the students.

The confusion incident to students passing to and fro in the laboratory is largely overcome. The student on entering the laboratory takes the drawer containing his cultures from the incubator to his desk, and proceeds with their study without further movement. When through with the day's work he returns the drawer to its proper place. He may also come in between periods, which is often necessary, and examine his cultures without interfering with the cultures of any one else. From the floor plan it will be seen that the desks for study are

in one part of the laboratory, while the apparatus for preparing media, etc., is conveniently grouped together in another part, so that students making media interfere in no way with those who are studying, and vice versa.

The laboratory abounds in many other conveniences for saving time and doing accurate work. Two nivellating tables, taking the place of the usual nivellating apparatus, occupy a position in the west part of the laboratory. They are covered with glass hoods, which keep out dust and at the same time admit light. Under these hoods inoculation of plates may be made with but small chance of infection.

A chemical cabinet, supplied with numerous stains, sugars, salts, alkalies, and acids necessary for use in the laboratory, together with balances and large scales, is placed handy to the tables for making culture media.

The laboratory library, occupying one corner of the room, separated from the main laboratory by a half-partition, is furnished with study tables, chairs, and best of all with an excellent collection of reference books. Spare moments, while waiting for media to sterilize, etc., are here utilized to good advantage, as well as the hours specially devoted to reference work.

A large cabinet for storing media has proved a great convenience. It is supplied with large drawers, in the bottom of which is a sliding box similar to those used in a library card cabinet. By moving this block backward and forward, either a small or a large number of tubes may be kept erect, avoiding the possibility of spilling or breaking, as is the case when loosely stored.

Wall shelves are located in convenient places, on which are found solutions of various disinfectants, alcohols, distilled water, measuring utensils, etc. A large supply of graniteware utensils of various sizes and forms are conveniently stored on shelves or in cupboards immediately under the tables used in making culture media.

Each student is furnished with a personal locker, detailed description of the construction of which was given in this journal in February, 1898. In this he finds a rack containing all bottles necessary for stains, two wire baskets, and two tin receptacles for storing test tubes, media, and cultures not put in the incubator, also a convenient holder for cultures while at work. (This is simply a block one and three-fourths inches thick with holes bored in one and one-half inches deep. The tubes may be placed in these without danger of falling over.) Inoculating needles, filter paper, jars containing cleaning mixture, complete the equipment of the locker. A small room off the general laboratory is used as general supply room, and wash-room for the janitor.

A very important adjunct to the laboratory, though not connected immediately with it, is the animal house. In it is found an abundant stock of guinea-pigs, rabbits, and mice. These, by continual breeding, with an occasional importation from other pens, maintain the stock. When an animal is inoculated it is immediately placed in a wire cage in a separate room, thus avoiding the infection of stock animals.

But better than all the practical apparatus and the conveniences of the laboratory, are the thoroughly practical methods employed in it, which will constitute the basis for a separate article.

## Influence of Light in Pigment Production of Bacteria.

In his *Manual of Bacteriology*, 1892, Sternberg observes that when *Bacillus mycoides roseus* is grown "upon the surface of agar, in the dark, a pink layer is developed, while in the light it is white." A similar statement is made by Schenk. Crookshank also observes that this bacillus, in the absence of light, produces a pink growth on agar; while McFarland refers to it as an example of bacteria which produce their pigments *only in the dark*. A number of other authors have been consulted, including Lehmann and Neumann, Lafar, Novy, Park, Abbott, Williams and Chester without finding any further mention of the matter. No author has been found who disagrees with those quoted, yet with the culture in my laboratory, which was obtained from a well-known and reliable government laboratory, and which appears in every other way perfectly typical, I have obtained diametrically opposite results. As these results are uniform, and have been observed year after year for a number of seasons, it seems desirable to call attention to the matter, since if my observations are correct many are being erroneously taught in this regard.

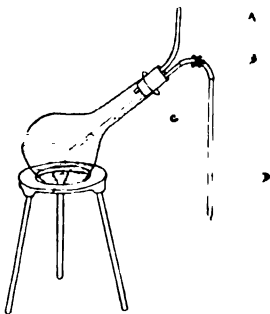
For the purpose of demonstrating to my classes the influence of light in the production of pigment by bacteria, it has been my practice to place chromogenes on similar media in an east window and duplicate cultures in a dark closet separated from the window only by the thickness of the boards of which the closet is constructed, the conditions as to temperature, etc., being identical. The result, with *Bacillus mycoides roseus*, on agar streak cultures, whether in warm or cold weather, has been to obtain a pale, yellowish red to rose color in the dark closet, and a deeper shade of a more brilliant hue and a more abundant pigment production in the parallel cultures in the window. On gelatin, which is slightly liquefied, the color in the window is still deeper than that on agar, reaching, after a few weeks' growth, a rich crimson. The latter medium has only been tried in winter, as it would be melted if thus exposed to the heat of the sun in summer.

Howard University.

W. W. ALLEGER.

## A Simple Form of Dropper for Use in Cutting Celloidin Sections.

A very convenient "dropper" for keeping the knife flooded with alcohol while cutting celloidin sections may be made, in a few minutes, from a 500 c. c. Florence flask and an iron tripod. The apparatus shown in the figure is so simple as hardly to need explanation. The tube D, which is drawn to a narrow opening at its lower end, must be, of course, a little longer than the tube C, to produce siphonal flow. The rapidity of flow is regulated by the screw-clamp B on the rubber connection. The weight of the alcohol in the flask will permit its being inclined at any desired angle, and, by having the tube C too short to reach the bottom of the flask, the flow of alcohol will cease while there is still enough of it left in the flask to prevent overturning. To start the flow, it is only necessary to blow into the tube A, as in an ordinary wash-bottle.



Allegheny College.

A. M. REESE.

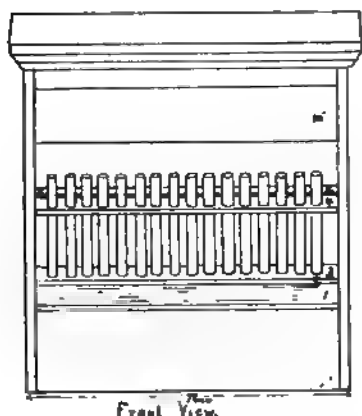
### Some Improvements upon Apparatus for Water Analysis.

Simple devices often lessen work and frequently with an increase in accuracy. Such devices are more than welcome in the water laboratory, where a large number of samples is handled and where so many of the processes are tedious and time consuming. It is with this thought in mind that the writers venture to present descriptions of two contrivances which have proved their usefulness in this laboratory, where many samples have been analyzed weekly in connection with the problems of the Chicago Drainage Canal. It may be that both of the schemes here presented are old, but if so, we are not aware of it; in any event they are good, and no harm can result from calling attention to them.

#### AN IMPROVED NESSLER CABINET.

Prof. Palmer of the University of Illinois is the inventor of the original form of cabinet and it has been in use in his laboratory at Champaign, Ill., for several years. The form of cabinet here described differs from the original in that it is constructed so that the sample may be moved along in front of the row of standards. It facilitates work and conduces to accuracy by enabling the analyst to see all of his standard at one time.

It is operated, as may be seen by referring to the accompanying figure, by



An Improved Nessler Cabinet.

placing the standards in a row on a glass shelf and passing light through them to the eye of the analyst by means of two adjustable mirrors. The sample to be estimated may be moved along near the row of standards until it is directly opposite the one corresponding most nearly to it.

The details are as follows: The standards are placed on the glass plate,

"1," which is supported by the shelf "2," and the bottoms of the tubes are kept from slipping by the block "3". The top of the tube is steadied by the perforated shelf "4". There should be accommodations for the full number of standards which is used, say eighteen or more. When the tube is in position, the mirror  $m'$  is so adjusted that the light from the proper part of the sky is reflected through the standards. The mirror  $m''$  is adjusted so as to reflect the beam at a convenient angle for the analyst to read. To avoid superfluous light the standards should all be placed in at once, thus filling all the holes. The sample to be estimated is placed on the glass over the slot  $S$  and held by the hand in a position parallel to the standards, then moved sideways till opposite the standard which is closest to it in value. It is convenient to place a board behind

the standards at X bearing figures denoting the amount of nitrogen or ammonia in the adjacent standard. If these are placed in reverse order they will appear in the mirror *m''* right side up. Owing to the flood of light through the slot *S* it is advantageous to place a blinder (consisting of a board 2 x 6 in., with one hole for the tube) over the end of the tube. The whole cabinet should be painted a dull black. The dimensions to fit our tubes ( $\frac{3}{4}$  x  $7\frac{1}{4}$  in.) are given with the figure.

#### DISAGREEABLE PIPETTING MADE EASY.

The simple device here described has been found of great value where it is necessary to pipette off large quantities of water for chemical examination, especially waters heavily polluted by sewage. The analyst is not only always in danger of sucking up a mouthful of the infectious, malodorous water, but, as is well known, the process becomes a very tiresome one when it is often repeated.

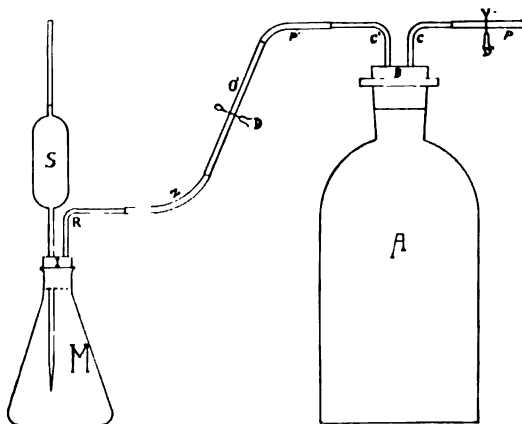
The apparatus constructed and in use in this laboratory consists of a large heavy glass bottle *A*, of about four liters capacity, fitted with a two-hole rubber stopper *B* clamped very firmly to the neck of the bottle by wire or otherwise, through which pass two glass elbows *C*, *C'*. To each elbow is attached a piece of rubber tubing *P*, *P'*. This is best held in place by wiring. Each piece of tubing is provided with a pinchcock *D*, *D'*. The piece of apparatus is now complete. The bottle with its fittings is next connected with the tap of the city water supply by means of rubber and glass tubing. The pinchcock *D* remains closed, while *D'* is opened at the same moment that the water from the tap is turned on. The water rushes in and compresses the air in the bottle. As soon as the bottle is one-third full of water the pinchcock *D'* is closed and the tap turned off at the same time. You now have a bottle two-thirds full of compressed air, obtained in a very easy way. The bottle is now taken to where the pipetting is to be done.

In our work the water to be examined is contained in 250 c. c. Erlenmeyer flasks, those with wide necks being preferred. A two-hole rubber stopper *I*, through which pass the pipette *S*, of desired capacity, and a small glass elbow *R*, is next fitted to the flasks. By carefully selecting flasks with uniform sized necks, one stopper will be sufficient. The glass elbow *R* is now connected with the bottle of compressed air by rubber and glass tubing *Z*, *O*. Next the stopper *I* is inserted into the neck of the Erlenmeyer flask, the jet of the pipette below the surface of the water, and the whole is held in place by the left hand. The pinchcock *D* is now opened very gently with the right hand and the air rushes into the flask *M*, forcing the water up into the pipette.

By a little practice one is able to regulate the amount of air necessary to fill the pipette. With the apparatus here described we have been able with a single charging to pipette off a liter of water. When the air is exhausted from the bottle, more water is run in and a new supply of compressed air obtained.

University of Chicago.

F. L. STEVENS AND W. G. SACKETT.



Apparatus for filling Pipettes in Water Analysis.

**LABORATORY PHOTOGRAPHY.***Devoted to Methods and Apparatus for Converting an Object into an Illustration.***PHOTO-MICROSCOPY OF METALS AS PRACTICED BY  
STEEL COMPANIES.**

When Henry Clifton Sorby of England reported the results of his study of the structure of meteoric and artificial irons at a meeting of the Iron and Steel Institute in 1864, in a paper "On the Microscopical Structure of Meteors and Meteoric Iron," very little attention was paid his report. After a lapse of twenty-two years the Institute requested Dr. Sorby, Dr. Percy, and Sir Henry Bessemer to decide what was the best way of illustrating a complete paper on the micro-structure of iron and steel. As the result of this investigation, Dr. Sorby presented two papers to the Iron and Steel Institute "On the Microscopical Structure of Iron and Steel." Since that time this means for the examination of metals and their alloys has increased in importance and value and within the last decade has become a recognized department in the testing laboratories of many industries, especially those of producers and users of iron and steel.

An equipment for the study of metal-microscopy or metallography consists of: a means for the preparation of the micro-section; reagents to "etch" the specimen; a compound microscope, which, in addition to the usual accessories, is equipped with a vertically movable stage and a means for "vertical illumination"; a light, strong, steady, and of uniform intensity, and a camera adapted to use with a microscope.

The piece of steel to be examined and photographed, having been machined to a suitable size for use on the microscope stage (the most convenient size and shape being either cylinders or cubes of about one-half inch), the surface to be examined is filed to a true plane and the polish is continued on successively finer grades of emery-cloth stretched over plane surfaces. It is well to change the direction of the application of the abrasive with each change in fineness, so that each set of scratches is at right angles with the previous

FIG. 1.—Pig Iron Polished in Relief.

set of marks. One set of marks must be entirely obliterated by the following set, and care must be taken to keep the polished face a plane surface or else difficulty will be met with in getting a flat field under the microscope. Following the polish on emery cloth the process is continued on sheets of muslin or canvas also stretched over plane surfaces and to which is applied the best Turkish flour-of-emery. Then follows a polish upon a similarly covered disk coated with a paste of calcined ammoniacal alum and powdered castile soap dissolved in water. Finally, the effacement of all scratches is completed upon a chamois skin stretched over a plane iron disk and coated with the best jeweler's rouge mixed with water. A properly polished specimen of steel, upon the completion of this process, should be microscopically free from all scratches and should look like a well-burnished piece of nickel or silver plating.

FIG. 2.—Steel after Polishing.

Where a large number of specimens are to be prepared the hand-process described is too slow and a polishing "head" is used, driven by power and so

arranged that four surfaces are at the command of the polisher. Generally these four surfaces are an emery-wheel or a carborundum disk of considerable fineness; a disk covered with canvas or linen duck for use with flour-of-emery or carborundum powder; a third disk covered with felt or billiard-cloth upon which is used alum and castile soap paste, and a fourth face for the chamois skin and rouge polish.

The polishing powders are most conveniently applied by means of bristle brushes to the revolving disks in the form of pastes made up with water.

FIG. 3.—Same as Fig. 2 after Etching.

The applications should be frequent rather than in greater quantities at longer intervals. For the sake of cleanliness the water supply arranged to trickle constantly on the disks of most grinding machines should be abandoned and the pastes applied wet enough not to need additional water.

To bring out the micro-structure of iron or steel it is generally necessary to apply an "etching" solution, although this is not always needed. Fig. 1 is a micro-photograph of a section of pig iron as it appears at the completion of the polishing process; no etching having been done. This is styled polishing in "relief." Fig. 2 is a micro-photograph of a piece of steel before etching, and Fig. 3 is the same piece after the application of a 25 per cent. solution of tincture of iodine in alcohol as an "etch".

The reagents used for etching iron and steel are various, depending mainly on the carbon-content of the steel and its heat-treatment. For mild and untreated steel, tincture of iodine, full strength, and in a 25 per cent. solution in alcohol, is used; for hardened steels 5 per cent., 10 per cent., and 20 per cent. solutions of nitric acid in alcohol are very satisfactory.

The polished surface of the micro-section should be freed from all grease and dirt by washing in alcohol and carefully dried. If the etching fluid is iodine, it is best applied with the tip of the finger or a camel's hair brush, keeping finger or brush lightly rubbing

FIG. 4.—Steel heated to 1200° C.

over the surface being etched and carefully watching the process, to prevent too deep an etching. The time necessary to properly etch a specimen cannot be definitely stated, as it varies greatly, ranging from a few seconds to a minute; depending upon the nature of the steel and the etching solution employed. A beginner will find it best to err on the safe side of stopping the process before the etching is complete; for should the examination show the structure insufficiently developed, the etching may be repeated until a desired result is obtained. A rule (with exceptions, of course) is to stop the action of the etch when specular appearance of the specimen has just disappeared and before the surface becomes dull and uniformly grey.

Having decided that the section is sufficiently etched, quickly hold the specimen under a tap of flowing water for a few moments, then remove all traces of moisture by the application of a few drops of alcohol, and finally dry it in a blast of air or by careful friction with very clean, old, and washed-out linen or cotton cloth.

Diluted nitric acid is used as an etching medium in case of hardened steel. With increase in hardness and working there is an increase in the fineness of the micro-structure of steel, to develop which it is best to use the more dilute solution of nitric acid. At the same time, the finer structures are the more difficult to develop and take a much shorter time to etch; hence it is particularly true that several short applications of the etch are more likely to produce the result sought for than one prolonged etching would. Examination between successive applications shows how the etching is progressing.

To etch with nitric acid pour a small quantity of the proper dilution into a suitable dish and, holding the cleaned

micro-section, face down, with pincers or a straight-nosed crucible tongs, dip the face to be etched at least twice into the etching fluid, immediately remove and let the action continue with face of section turned up, so that the action may be watched. When it is complete (two seconds is often enough) hold in stream of water and dry as described before. If examination shows etching to be incomplete repeat the process; if too deeply etched, repeat the polishing.

Various devices for temporarily mounting specimens, especially for those of irregular shape, are supplied by dealers, but none has been found more convenient than a small one and one-half inch square of ground glass upon which is a lump of beeswax the size of a hazel nut. It need not necessarily be ground glass, but generally an identification-mark is needed and then the ground surface is very convenient to write on.

To mount an irregular

FIG. 5.—Same Steel as Fig. 4 heated to 630° C.

FIG. 6.

section so that the etched surface is parallel to the ground glass base, a microtome is a great convenience (M Fig. 8). Projecting from a cylindrical metal base three inches in diameter, is a threaded upright three inches high and one and one-half inch in diameter. A cylindrical nut or collar three inches high and two and one-half inches outside diameter screws on the threaded upright. A small circle of chamois skin is placed on the top of the threaded upright to protect the etched face of the micro-section. To mount a section place it face down on the chamois skin, press upon the upper projecting portion the lump of beeswax and upon this place the ground glass (ground surface down). A few revolutions of the collar will cause the glass to rest upon the upper edge of the collar and the adhesion of the glass and beeswax to the specimen may be made complete by slowly turning the collar down with one

hand while keeping the glass base in close contact with the collar-top with the other hand. In this manner, no matter how irregular the section, the parallelism of the etched surface and the glass base may very quickly and accurately be obtained.

The magnification used in ordinary investigations ranges from 30 to 100 diameters. The micrographs accompanying this article are uniformly 65 diameters. Photo-micrographs 4 and 5 are from the same bar of medium carbon steel etched with tincture of iodine for ten seconds. The difference in the size of the

FIG. 7.

grains of the two specimens is caused by the degree of heat to which the two sections were subjected; 4, having been heated to 1200 degrees Centigrade and 5 to 630°C.

When the examination shows that the specimen has been properly prepared the observer selects a spot representative of the micro-section under consideration. Here the temptation arises to take that which is unusual rather than that which is characteristic or to photograph "freaks" instead of normal structures. Unusual structures frequently occur in metals or alloys that have been subjected to a variety of treatments in the way of reheating or reforging. Figs. 6 and 7 were taken from the same micro-section of steel within a circle of less than one-half inch radius, and show a variety sometimes found in a single specimen.

Arranged upon a well-equipped optical bench, in the following order, are these parts (see Fig. 8): a source of light, the best (after sunlight) being an automatic-feed arc lamp (A), surrounded by a suitable case, with an opening at

one side, in front of which is arranged a system of condensing lenses (B), similar to those used in high-grade projection lanterns; a cell filled with a saturated solution of alum (C), to absorb the heat rays; a double-convex lens (D), to further concentrate the beam of light; a holder for color and ground-glass screens (E); an iris diaphragm and a photographic shutter (F) with pneumatic release; and probably another double convex lens (G), all of which are so secured to the optical bench as to be movable in a horizontal direction for the purpose of focusing the light on the vertical illuminator. All the members of this system are also adjustable vertically, so they may be brought into alignment with the source of light.

The beam of light leaving the lens system enters the body of the microscope (H, whose tube is vertical), through the opening of the vertical illuminator, inserted between the objective and the main tube. The vertical illuminator con-

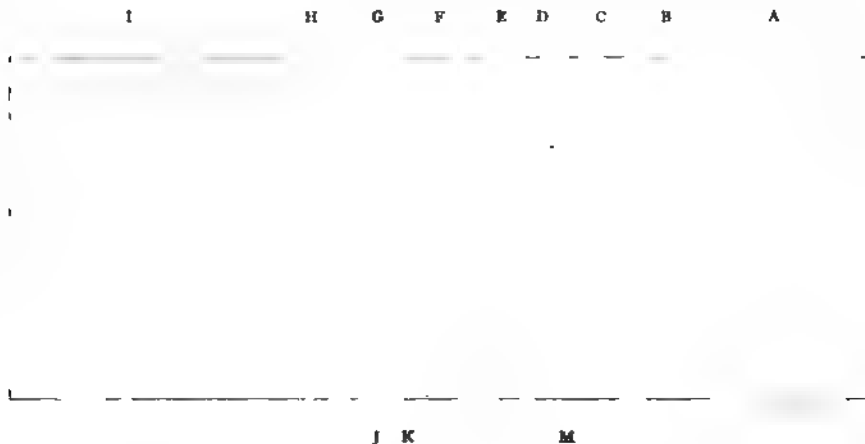


FIG. 8.—Optical Bench for Photo-microscopy of Metals.

tains either a plain glass disk reflector or a total reflection prism, which changes the direction of the horizontal beam of light to a perpendicularly descending beam. The objective of the microscope acts still further as a condenser and an exceedingly well-illuminated spot is thus secured on an opaque body.

The camera used in the photomicrography of metals is generally one adapted to 4 x 5 inch plates, and is so arranged that it may be swung out of the observer's way while an examination is made. The cut shows it in this position. When the part to be photographed is selected the eye-piece of the microscope is removed, the collar (K) to connect the microscope and the camera is placed over the end of the tube and the eye-piece is replaced within the collar. The camera is swung into position vertically over the microscope, the front board of the camera is lowered until its collar telescopes with that on the microscope. The upper, or ground glass, end of the camera is moved up or down until the circle of the projected image is the size desired. The ground glass of the camera has cemented to its center a large cover-glass, giving a clear-glass spot in the middle of the image. The final focusing for sharpness and clearness of the micro-

image is done with a focusing eye-piece (J) set on the clear spot in the ground glass and is obtained by the use of the slow-motion screw of the microscope.

Slow isochromatic plates have proven the most satisfactory for all around use, as they give the color values without the use of a color screen, thus avoiding long exposures, with their attendant ills, vibration of the optical bench and irregularity in illumination.

With an electric arc light of about 2000 candle power, a system of good condensers, a prism reflector, and using a magnification of 65 diameters, an exposure need not be longer than twelve seconds. Developed with ordinary pyro developer, a negative giving good, contrasty prints should be obtained. A glossy paper should be used to reproduce a micro-negative.

If it is desired to preserve the micro-section it should be coated with a solution of paraffin in benzole and placed under a bell jar or in an air-tight case containing a vessel filled with calcium chloride or other hygroscopic substance.

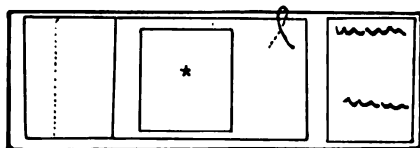
To be of practical value a complete record should be kept of the history of the metal examined, embracing its chemical composition, its treatment with regard to heating and forging, the results of the physical tests, nature of the etching solution employed, magnification and illumination used, and any other related facts that may be considered of importance. Special pains should be taken to clearly state the location of the micro-section in the original piece of metal and whether the surface photographed is longitudinal or transverse. Card index files are published, which make possible the combination of the mounted micro-photograph with all of the data mentioned.

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### Double Mounting for Whole Objects.

It is frequently desirable to have a small object mounted in such a way as to be readily studied from both surfaces. The plan of mounting on a large cover-glass with a smaller slip used as a cover has been in use for some time. The usual method was to fasten the larger, or bottom, slip upon an ordinary microscopical slide by bits of gummed paper, and when it was necessary to examine the lower surface of the object the bits of label were cut in two, the thin glasses turned over, and then replaced with other stickers. This left the object in



danger of injury while unmounted, and was clumsy. I want to suggest a "new wrinkle," which I have never seen used or described, although it may be old to some microscopists. Gum one end only of the larger cover-slip to the slide by a piece of label, or better, of sticking plaster, and let this serve as a hinge, so that the covers can be turned back, past the end of the slide. A small wire clip, half of a Niagara clip for instance, is then used to hold the covers firmly to the slide, in either position. This is a safer, quicker, and neater method than the old one.

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## METHODS IN PLANT PHYSIOLOGY.

## III.

## RESPIRATION—Continued.

3. **The Respiratory Ratio in Starchy and Oily Seeds.** The ratio of the volume of oxygen inhaled to the volume of the carbon dioxide exhaled is usually expressed by the formula  $\frac{CO_2}{O} = 1$ , which means that the volume of the oxygen absorbed is equal to the volume of the carbon dioxide given off. This ratio probably holds true where the oxygen is used only for respiration, but in the germination of oily seeds part of the oxygen is used to convert the oil into starch, thus causing the absorption of oxygen to be greater, volumetrically, than the evolution of carbon dioxide. The difference may be demonstrated in the following manner: Place about 20 g. of imbibed seeds of wheat or barley in a 500 c. c. Erlenmeyer flask, connect it with a vessel of mercury by means of a continuous piece of glass tubing; prepare a precisely similar piece of apparatus, using seeds of hemp or flax. A thermometer is inserted in the rubber stopper in the mouth of the flask and all joints tightly sealed (Fig. 8). By gently warming the flask a little air is driven out and mercury is drawn into the glass tube; in this way the lower portion of the tube is filled with mercury. When the flask has cooled to room temperature mark the position of the mercury with a thread or strip of gummed paper, at the same time recording the temperature of the flask. Keep the apparatus from direct sunlight in a room of even temperature. The final observation should be made eighteen to twenty hours later; at that time bring the flask to the same temperature as at the beginning of the experiment, and observe the point to which the mercury column extends. The starchy seeds will cause an increase in the volume of gas, while the oily seeds will show a decrease in the volume of the gas in the flask.

FIG. 8. A means of demonstrating the respiratory ratio. The horizontal tube is about 60 cm. long and terminates in a dish of mercury.

4. **Intramolecular Respiration.** The evolution of carbon dioxide from plants deprived of oxygen may be demonstrated successfully by either of the following methods:

(a.) Fill a test-tube with mercury, cover the mouth of the tube with a small disk of cork and invert the tube over a dish of mercury. The disk of cork can be easily slipped off, and holds the mercury better than the finger. Remove the

seed-coats from three peas (*Pisum sativum*) which have been soaked from 4 to 6 hours, and carefully pass the peas into the tube of mercury without allowing any air to enter. The peas will at once pass to the upper end of the test-tube; at the end of 36–48 hours they will have evolved a quantity of carbon dioxide equivalent to their own volume. The gas may be proved to be carbon dioxide by absorbing it with potassium hydroxide. With a curved pipette filled with water to the extreme tip, introduce 1 or 2 c.c. of water into the test-tube, then with the forceps pass in a small piece of solid potassium hydroxide which dissolves almost immediately; shake the tube gently for a few minutes and the carbon dioxide will be absorbed.

(b.) Set up such a series of bottles as shown in Fig. 2, leaving off F and attaching a hydrogen generator to A. B, D, and E contain a saturated solution of barium hydroxide; A, a 20 per cent. solution of potassium hydroxide; C, a few pea or corn seedlings interspersed with pieces of damp filter paper. Allow the hydrogen to flow through A, B, and C alone until all air is removed from the apparatus, then connect C with D and E. After 30–45 minutes, depending on the flow of hydrogen, D will contain a white precipitate of barium carbonate, proving the evolution of carbon dioxide in the bottle containing the seedlings, even though no oxygen be present.

5. **To Demonstrate the Evolution of Heat by Respiration.** The extremely active respiration of germinating seeds produces a very perceptible amount of heat, the important point in conducting the experiment is to measure the heat before any is lost. Proceed as follows: Place 100 c.c. of a 10 per cent. solution of potassium hydroxide in a salt-mouth bottle of about 500 c.c. capacity, set a funnel in the mouth of the bottle and put about 500 c.c. of softened peas into the funnel, interspersing pieces of wet filter paper among the peas; cover the bowl of the funnel with a thick layer of cotton batting to prevent radiation of heat. Over the funnel set a bell-jar with a tubulature above, and through the tubulature insert a thermometer graduated to fifths of a degree Centigrade, immersing the bulb of the thermometer in the peas, and filling the tubulature with cotton.

Make an exactly similar preparation for a control, using wet sawdust in the place of peas. Place the two preparations side by side in a place where they will not be exposed to direct sunlight and keep the temperature as constant as possible. Allow the two preparations to stand for 4 to 6 hours before making an observation. Final observations should be made within 24 hours, otherwise the growth of bacteria in the sawdust may raise the temperature of the control. The potassium hydroxide solution prevents any excess of carbon dioxide from interfering with the respiration of the seedlings.

NOTE.—To prepare the barium hydroxide solution used in the foregoing experiments, place 50–75 g. of dry barium hydroxide in a bottle holding 2 or 3 liters, fill the bottle with water, and shake; there should be enough residue to keep the solution saturated. Filter off a part of the solution into another bottle and keep it tightly stoppered; use from this bottle. After using the solution it may be returned to the large bottle, and used again after filtering.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### VIII. ABNORMAL CONSTITUENTS OF THE URINE—Continued.

#### SEDIMENTS.

The examination of the sediment in urine is now comparatively easy since the centrifuge has come into common use. Fill both tubes of the centrifuge with urine and revolve them in the instrument for three minutes at a speed of 1000 revolutions per minute. Decant the urine, and with a pipette transfer some of the sediment to a clean slip. Lay in the drop of urine a hair and over all apply a clean cover. Examine with a  $\frac{2}{3}$ -inch and later with a  $\frac{1}{4}$ -inch objective.

There are two kinds of sediments—chemical and anatomical.

The chemical sediments exist in normal urine usually in solution. Abnormally they may appear as amorphous or crystalline when precipitated, due to changes in the urine or to abnormal quantity of them.



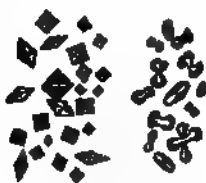
Uric Acid Crystals.

Uric acid crystals appear as brightly colored, yellowish red crystals, rhombic in form in general. They cluster together, and in appearance resemble a pile of loose shingles, and as they are always more or less colored cannot be mistaken for anything else. If uric acid crystals are found in the urine within four hours after it is voided it should be considered

pathological. Such a condition indicates that it may precipitate in the kidneys or bladder and give rise to calculi and gravel. Uric acid crystals appear in the urine in high fevers and in the so-called uric acid diathesis.

The urates of potassium, ammonium, and calcium are met with in the sediment. They are usually amorphous in form, with occasional minute star shaped or needle shaped crystals in the sediment. The color of the mixed deposit is usually slightly reddish in color and granular. These deposits are usually indicative of a febrile state and also occur in wasting diseases.

Acid Ammonium  
Urate.



Calcium Oxalate.

Calcium oxalate crystals are characteristic, appearing like the backs of envelope with lines from the corners to the center. Occasionally they appear dumb-bell in shape. Oxyluria is indicative of some disturbance of the nervous system which may be due to impairment of the blood, imperfect respiration, etc.

Phosphate crystals and the amorphous deposits of the combinations of phosphoric acid both occur in urine that is alkaline or at the neutral point towards alkalinity.

Triple phosphate crystals appear in star shaped rosettes or characteristic coffin shaped crystals. Calcium phosphate crystals are somewhat similar to uric acid crystals, but smaller, more delicate and devoid of color. These crystals indicate an alkaline urine which is abnormal in itself; quantities indicate a general lowering of vitality. If constantly abundant in freshly voided urine, calculus should be suspected.



Triple Phosphates.



Leucin. Tyrosin.

Leucin and tyrosin are found in the urine, the former in the form of globules which are nearly as brilliant as oil globules. It is insoluble in ether, which differentiates it from oil globules. Tyrosin crystallizes in very fine needle shaped crystals, arranged like sheaves of grain. They may be classed among the rarer sediments. In cases of extensive suppuration, leucin and tyrosin may appear in the urine in large quantities, partially in place of urea. They are also found in cases of acute yellow atrophy of the liver and in phosphorous poisoning.

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Harvey Medical College.

## The Technique of Biological Projection and Anesthesia of Animals.

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### VI. PROJECTION MICROSCOPES USING ELECTRIC ARC OR OXYHYDROGEN LIGHT.—Continued.

This lamp may be used on either incandescent or arc currents, the former usually of 110 volts or less, the latter with a voltage of about 2000, and on either the direct or alternating currents of either system. A fixed rheostat suited to the current or an adjustable rheostat, which is more convenient, if the lamp is to be used on different currents, is always necessary. A flexible wire cable or heavily insulated copper wire (about No. 12) is connected with the binding-posts in the base board. One of these cables runs to one pole of the fuse-box of the building or to some other point where it is possible to connect with the heavy feed wires which supply the current. The other cable connects with one binding-post of the rheostat and from the other binding-post of the rheostat another cable connects with the other heavy feed wire in the fuse-box or elsewhere, as above described. Do not attempt to take a current from an incandescent light socket. The switch should remain open until all connections have been made, and before closing the switch test the working of the feed-wheels and separate the points of the carbons about a half turn of the wheels. Look through the colored glass window in the door of the lantern body, after the switch is closed, and turn both feed-wheels at the same time until the carbons come into contact and the arc is produced, and then *instantly* turn the feed-wheels backward about a quarter turn

or 90°. If an alternating current is used, a more or less pronounced humming sound will be heard, but lecturer and audience soon become accustomed to it. If the direct current is used, care must be taken to so connect the cables leading from the switch to the lamp that the horizontal carbon will be positive. An easy method for determining the positive carbon is to allow the lamp to burn for a moment or two, then open the switch and observe the carbons. One will be seen to be more luminous because more highly heated and it is the positive carbon. If it is also the horizontal carbon the connections are correctly made; if not, detach at the lamp and change the ends of the cables to the other carbon. It is well, at first, to exercise special care lest one put his body in a circuit by touching the two carbons, or their holders, or other uninsulated parts at the same instant. A 110 volt current running through one's body is not likely to produce serious results, if indeed it has any effect other than to convince one that it is not a desirable mental stimulus for one who is lecturing. Currents from arc systems are not as desirable as from incandescent systems on account of the danger attending their use.

When using a direct current, the horizontal carbon, or the one which furnishes the available light, must be positive. To test it, open the switch, after the lamp has been lighted about a minute, and look at the points of the carbons. One carbon will be seen to be more heated and so remain red longer than the other. The hotter carbon is positive and, if it is not the horizontal one, reverse the wires at the lamp. With alternating current the carbons are equally luminous.

Having tested the lamp and obtained a good light, proceed as follows to adjust the lamp and microscope for projecting. Fill the water tank with clean water from which the air has been expelled by boiling for a few minutes and then cooled in an air tight fruit can. Leave a small air space in the top of the tank to allow for expansion as the water becomes heated during use. See that the condensers are clean and set squarely in their places and that all of the optical parts are in the same optical axis. Set the lamp so that the point of the vertical carbon will be three inches from the adjacent face of condenser number one, and adjust the tip of the horizontal carbon as accurately as possible in the optical axis of the lenses. Put on a low power objective, e. g., a  $\frac{3}{4}$ -in. or 1-in., remove the sub-stage condenser and amplifier, set the microscope carrier at such a position that an object on the stage of the microscope will be about twelve inches from the vertical carbon, measured along the optical axis. These measurements are approximate and intended simply as a guide in making the first test of the instrument. Place a mounted object, or a slide on which an ink line has been drawn with a pen, on the stage and turn on the current at the switch. Strike the arc, as directed above. As the available light is derived from the horizontal carbon, care must be taken that the vertical carbon is not too high so as to intercept the light. Focus the objective carefully and proceed as follows to test the light for its maximum efficiency. If the circular field on the screen is not evenly illuminated, raise or lower the entire lamp or move the arc to the right or left until the illumination is central. Slide the microscope carrier slowly toward or away from the light and clamp it at the position giving

best results, and record the distances of lamp and microscope from the condenser for future reference. Now slide the lamp carrier slowly toward or from the condenser, noting the point at which the best light is obtained. Keep the arc as steady as possible by due attention to the feed. Time spent in thoroughly testing the apparatus is not wasted, if records of the best results are made so that the same arrangement of parts can be made at any subsequent time by measurements.

A. H. COLE.

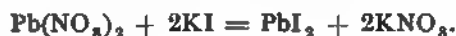
University of Chicago.

## MICRO-CHEMICAL ANALYSIS.

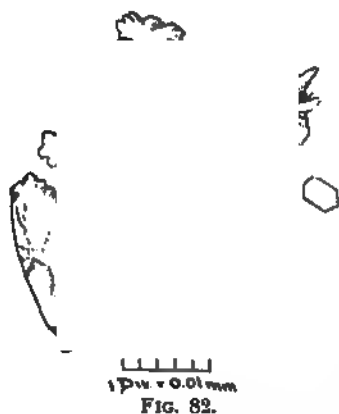
### XX.

#### SILVER GROUP CONTINUED—LEAD.

##### III. Potassium Iodide added to solutions of salts of Lead precipitates Lead Iodide.



*Method.* To the test drop, which should not be too dilute, add a tiny fragment of the reagent. A bright yellow precipitate is at once formed in a circular



band about the reagent fragment. The circle gradually becomes larger and larger and at its outside circumference beautiful hexagonal plates appear. These plates and flakes of lead iodide appear greenish or brownish yellow by transmitted light, sometimes even gray, according to their thickness. By reflected light lead iodide plates glow and glisten and display the iridescent colors of thin films, an extremely characteristic feature of this salt.

These hexagons of lead iodide do not belong, according to Behrens, to the hexagonal system as usually stated, but are probably orthorhombic, this view being based upon the

fact that the hexagonal plates of lead iodide are dichroic.

The usual forms assumed by lead iodide are shown in Fig. 82.

*Remarks.* An excess of the reagent must be avoided, otherwise the precipitate at first formed will be dissolved because of the formation of a double iodide of the composition  $\text{PbI}_2 \cdot 2\text{KI} \cdot x\text{H}_2\text{O}$ .<sup>1</sup> Not infrequently colorless crystals of this double iodide will be seen in the immediate neighborhood of the reagent fragment. The addition of a drop of water will usually cause the decomposition of the double salt and a precipitation of the normal iodide.

Double iodides of lead with many elements are known, most of them crystallizing readily<sup>2</sup>, but it is not often that there will be a sufficient separation of

<sup>1</sup> Brooks, Chem. N., 1898, 191.

<sup>2</sup> See Mosnier, Ann. chim. phys. (7) 12, 374. Comptes rend. 120, 444.

these interesting salts to interfere in any way with the detection of lead.

From neutral solutions containing lead in the form of lead acetate, potassium iodide will generally precipitate, in addition to the normal iodide, basic iodides of variable composition, such as,  $\text{PbI}_2 \cdot \text{PbO}$ ;  $\text{PbI}_2 \cdot 2\text{PbO}$ .

In most cases it will be found advantageous, before adding the reagent, to acidify the test drop with acetic or with nitric acid.

Lead iodide can be recrystallized from hot water, best if acidified with nitric or acetic acid. On cooling, large, beautifully formed hexagons separate. A large drop of water is necessary in order that good results may be obtained.

Heated with hydrochloric acid lead iodide dissolves, and on cooling crystals of the normal iodide  $\text{PbI}_2$ , a chloriodide,  $\text{PbCl}_2 \cdot \text{PbI}_2$  or  $2\text{PbCl}_2 \cdot \text{PbI}_2$  (or both), and the normal chloride  $\text{PbCl}_2$  separate. The chloriodides appear in the form of needles of a faint yellow color.

Too much nitric acid in the water employed for recrystallizing the precipitate of lead iodide will cause partial decomposition and consequently the separation of the colorless octahedra of lead nitrate.

Silver iodide separates as a yellowish amorphous mass insoluble in hot water and in hot nitric acid.

Mercuric iodide takes the form of ruby red rhombs. Mercurous salts acidified with nitric acid usually give in addition to the heavy precipitate of mercurous iodide the ruby colored rhombs of the mercuric salt.

If cuprous salts are present a white granular precipitate of cuprous iodide is formed and iodine is set free.

Thallium is precipitated as an exceedingly fine granular precipitate.

Antimony and bismuth salts interfere with the reaction for lead. These elements yield with potassium iodide, double iodides which separate in neat, well formed crystals (see Antimony and Bismuth). Solutions containing lead, antimony and bismuth, when treated with potassium iodide, yield a dark reddish brown, sandy precipitate wholly unlike in appearance anything obtained with the different elements alone. Boiling the mixed product with water will generally cause a partial decomposition, and on cooling hexagons and irregular plates of lead iodide will appear. Solutions of lead and bismuth yield orange red disks and plates.

Selenium and tellurium under certain conditions are precipitated as dark reddish brown iodides.

#### *Exercises for practice.*

To a test drop containing  $\text{Pb}(\text{NO}_3)_2$  add KI. Study the preparation, then add a drop of water and heat to boiling. After the drop has cooled study it again. Repeat the experiment, but this time use an excess of KI. Try again in acidified solutions.

In like manner test a preparation of  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ .

Make a preparation of  $\text{PbSO}_4$ . Draw off the mother liquor, add to the sulphate residue a drop of water, acidify with nitric acid, then add a fragment of KI. After a few seconds examine the preparation.

Make a mixture of Pb and Ag, test with KI. Then try in turn mixtures of Pb and Sb; Pb and Bi; Pb, Sb and Bi; Pb and Cu; Pb and Sn.

## LABORATORY OUTLINES.

### For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

#### A SERIES OF FORMS TO ILLUSTRATE THE EVOLUTION OF SEX.

XIV. *Sphærella pluviæ* (Flotw.) Wittr. (*Hæmatococcus*). Order, *Proto-coccales*. Family, *Volvocaceæ*.

*Sphærella* may be found growing in rain water, drain tiles, roof gutters, pools, or ponds. It is unicellular and green in color or sometimes a bright red. If a culture is once obtained, it may be preserved on a limestone or sandstone rock. Put the rock into the water containing the alga and after some time take it out and lay it away. Whenever material for study is required the rock need only be placed in fresh rain water, when a new crop will soon appear.

1. With a medicine dropper mount some water containing *Sphærella* and examine under low power. Under high power study the large, green, spherical cells in the resting condition. Draw. Notice the green and red coloring matters—chlorophyll and hæmatochrome.

2. Draw an individual divided into two, and one divided into four cells. How does the division take place as regards the cell wall? Compare with *Pleu-*



FIG. 4.—Life Cycle of *Sphærella*.

rococcus. Look for an individual in which the four cells are ready to break through the old cell wall. The four cells form four free-swimming *Sphærellas* which have very loose cell walls.

3. Study the active individuals. Describe the shape, color, cell contents (especially the chloroplasts and pyrenoids), and the flagella. The flagella branch out from a clear body in the pointed end of the cell and pass out through two extremely minute openings in the cellulose wall.

4. Study and describe the movement. Which end is directed forward in swimming? How long does it take an individual to pass across the diameter of the field? Suppose the diameter is three-tenths of a millimeter, how long would it take the plant to travel thirty centimeters or one foot? Is the motion rapid or slow? How many times its own diameter does an individual move in one second?

5. The flagella and other parts may be seen more clearly by adding a small drop of iodine solution to the water at the edge of the cover-glass. What hap-

pens? How long are the flagella, compared with the size of the body? Look for the nucleus. Notice the protoplasmic strands which pass from the cell-body to the wall.

6. Notice the division of labor in the organism and designate the function of the following organs: *a*, cell wall; *b*, flagella; *c*, chloroplasts.

7. Make a diagram in the notes showing the life cycle of *Sphærella* when reproduction takes place by the formation of non-sexual zoöspores. (See Fig. 4 a.)

8. Look for individuals divided into six or eight cells. Draw. When these escape they are smaller than the four and at first have no cell walls. These zoöspores are said to conjugate and form zoözygospores as is the case in *Sphærella buetschlii*. Careful observations should be made in order to discover such a process. In case conjugation takes place the life cycle during this stage may be represented as in Fig. 4 b.

#### XV. *Pandorina morum* (Muell.), Bory.

Order, Protococcales. Family, Volvocaceæ.

*Pandorina* occurs in small pools of water, and is often very abundant in summer, coloring such pools a bright green. The individuals consist of a free-swimming colony of sixteen cells, and are more or less globular or oval in outline.

1. Mount some of the colonies in water and examine under low power. Notice the active movement. Draw a colony under high power. If they cannot be followed because of their active movements, add a drop of carbolic acid water.

2. Notice the details of an individual cell of the colony; the two flagella, the red eyespot, the transparent spot in the outer end of the cell, and the chloroplast with a pyrenoid.

3. Study and draw colonies in stages of division. Each of the sixteen cells divides until each forms a group of sixteen new cells, then the gelatinous envelope dissolves and the sixteen daughter colonies are set free. This is the normal method of vegetative propagation.

4. Sexual reproduction. Look for colonies in which the cells are separating as isolated zoöspores. These are the gametes which are very much alike, but are of various sizes.

5. Watch for conjugating forms. Conjugation takes place between two gametes of equal size, or between a larger and a smaller one. The process is complete in a few minutes. Draw stages observed, and also mature zygosporos. The difference in size of the conjugating gametes is of special importance, since it is the first step in the evolution of two specialized gametes, the oösphere and spermatozoid.

6. NOTE.—*Pandorina* is well preserved in water with carbolic acid, and large quantities may be collected at the proper season, showing the various stages of the life cycle. Cultures can also be obtained in the laboratory from dry zygosporos.

JOHN H. SCHAFFNER.

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The fifty-second annual meeting of the American Association for the Advancement of Science and affiliated societies was held at Pittsburg, Pa., June 28th to July 3d and was one of the best attended and profitable gatherings of these bodies. There were about seven hundred and fifty members present and over three hundred and sixty-five papers were presented. It was characteristically a working meeting. Too much cannot be said in appreciation of the hospitality of the local committee and citizens of Pittsburg in providing entertainment for the visitors and in showing them the, to the majority, unrealized greatness of their vast industries and institutions.

The American Microscopical Society met at the lecture hall of the Phipps Conservatory June 27-28th. An extremely interesting program was presented and the members present were especially impressed with the real value of participating in such a meeting. The following officers were elected for the ensuing year; the secretary, treasurer and custodian hold over, being elected for three years: Pres., E. A. Birge, Univ. of Wis., Madison, Wis.; 1st Vice-Pres., Wm. H. Seaman, Washington, D. C.; 2d Vice-Pres., A. M. Holmes, Denver, Colo. Elective members Executive Committee: L. B. Elliott, Rochester, N. Y.; M. J. Elrod, Univ. of Mont., Missoula, Mont.; F. S. Hollis, Yale Medical School, New Haven; Asst. Sec'y., R. H. Wolcott, Lincoln, Neb.

The custodian of the Spencer-Tolles fund, Mr. Magnus Pflaum, reported that he has plans to raise the fund to \$5000 and the society endorsed the plans. Everything that could be done for the comfort and convenience of the members was done by the local committee and our especial thanks are due to the chairman, Mr. Magnus Pflaum, who provided solid silver pins bearing a microscope and Pittsburg, 1902, in relief. Mr. Pflaum had also the members of the society as his guests at a very enjoyable theatre party. Dr. Holland, director of the Carnegie Institute, extended many courtesies, which were highly appreciated.

The September JOURNAL will be a special number devoted exclusively to short original articles. It will be one of the most valuable numbers of the year, embracing many practical methods and improved apparatus for a variety of purposes.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Keinitz, Gerloff F. Neue Studien über Plasmodiesmen. Ber. d. deutsch. bot. Gesell. 20: 93-117, pl. 4, 1902.

In this paper the writer gives the results of his studies upon protoplasmic connections in a large number of plants belonging to all groups from the algæ up to the flowering plants. The following method is recommended: The moss leaves, filamentous material or sections were treated with iodine solution and then allowed to swell for about twenty-four hours in weak sulphuric acid (1 part  $H_2SO_4$  and 3 parts water), after which they were stained in methyl violet (0.1 g. methyl violet + 80 c. c. water to which is added an equal volume of the weak sulphuric acid). The iodine and sulphuric acid not only serve as fixing agents, but also act as mordants.

Previous investigations have made it seem very probable that protoplasmic connections are universally present in the phanerogams. The present work shows that the connections are as uniformly present in the lower groups, the filamentous algæ forming, perhaps, an exception; but in such algæ, each cell has a large degree of independence in nutrition and reproduction, so that the absence of the connections need not occasion surprise.

The suggestion is made that the connections may not be derived wholly from the *Hautschicht*, but that they may consist of trophoplasmic core covered by *Hautschicht*. The theory that the connections conduct stimuli and materials is supported, but the writer believes that the whole subject still needs investigation.

C. J. C.

Gager, C. S. The development of the pollinium and sperm cells in *Asclepias Cornuti*, Descaignes. Annals of Botany, 16: 123-148, pl. 7, 1902.

For the fourth time we call the attention of readers of the JOURNAL to a paper dealing with the pollen mother cells of *Asclepias*. The first paper, which was merely mentioned by title, was by Wm. C. Stevens, who in discussing spindle formation, incidentally says that the pollen mother cell gives rise to four microspores arranged in a row; but the statement is made as if he were referring to an accepted fact and not as if he realized that it was a real contribution. Strasburger and Frye, whose work appeared almost simultaneously, made it plain that the mother cell gives rise to four microspores. The present paper, which is accompanied with good illustrations, traverses about the same ground and arrives at the same conclusions.

C. J. C.

Shibata, K. Die Doppelbefruchtung bei *Monotropa uniflora* L. Flora, 90: 61-66, 1902.

The fertilization of the egg and polar nuclei of *Monotropa uniflora* is practically the same as in all other forms in which double fertilization has been observed. The sperms are vermiform when they enter the embryo-sac, but

shorten themselves as fusion progresses. The second sperm attaches itself to the upper polar nucleus; these two are soon joined by the lower polar nucleus, and the three fuse simultaneously. The endosperm nucleus divides before the fertilized egg shows signs of division. This is true of all forms except *Naias*, in which, according to Guignard, the egg divides first. The two deeply staining bodies observed by the reviewer in the pollen tubes of *Silphium* and *Erigeron* were also seen in *Monotropa*. Their origin was not determined. The article is illustrated with ten excellent figures.

W. J. G. LAND.

Chicago.

Ducamp, L. Recherches sur l'embryogénie des Araliacees. Ann. Sci. Nat. Bot. Ser. VIII. 15: 311-402, pls. 6-13, 1902.

In the Araliaceæ, as a rule, there is only one archesporial cell in the nucleus of the ovule. This cell gives rise

to a tapetal cell and a sporogenous cell which is the megaspore mother cell. The megaspore mother cell may develop directly into the embryo-sac or may give rise to an axial series of three or four potential megaspores. In *Fatsia Japonica* a case is figured in which the middle cell of an axial row of three cells had divided longitudinally. In another case the mother cell had divided transversely and each daughter cell had then divided longitudinally. Similar divisions seem to occur in *Aralia racemosa*. In one case the mother cell is described as having given rise to an axial row of four cells, the upper three of which had subsequently divided longitudinally. The writer states that the division of the nucleus of the mother cell sometimes takes place without the formation of cell walls, but the figures are very small and may bear another interpretation. The megaspore nearest the chalaza usually functions, but the middle one in the axial row develops at the expense of those above and below. Double fertilization was looked for, but nothing definite was found. The development of the embryo and appearance of tissue systems are described in detail.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole, 55 S. Marengo Avenue, Pasadena, Cal.

Bogdanow, N. O proisschoshdenii i snatschenii eosinofilnoi sernisstossti i ob otnoschenii eja k prozessy krowetworenija [ueber die Entstehung und Bedeutung der eosinophilen Körnung und ihre Bedeutung für die Blutbildung.] Mang. Diss. Moskwa 188 pp., Tafn 2, 1899.

Air dried smear preparations of blood, sputum, pus, etc., are fixed in equal parts of ether and alcohol (Nikiforow's mixture) for 20-30 minutes, then stained for 3-5 minutes in a 1 per cent. solution of eosin in 60 per cent.

alcohol which has been diluted one-half with distilled water just before using. These are washed off in distilled water or merely dried with blotting paper and double stained in a saturate aqueous solution of methylen blue, which too is

diluted one-half with distilled water before using. Then these are rinsed in water, dried in the air and mounted in Canada balsam. To bring out the granule stain most clearly, Mandybrer earlier recommended putting the preparations in a 3 per cent. solution of copper sulphate to which has been added enough ammonia to dissolve the incipient precipitate.

Investigations were carried out chiefly on the edge of the liver of the animals used (frog, dog, cat, ram, guinea pig, rabbit, rat, monkey). All experimental work was carried out on dogs. To obtain bone marrow of warm-blooded animals, the following procedure was adopted: The animal was cut rapidly across the throat (severe anæmia of marrow takes place in stabbed animals), the thigh bone is freed at one cut from all its soft and hard bonds, and the upper epiphysis sawed off at about the level of the spongy bone tissue together with two succeeding pieces of about a finger's breadth in length. The bone segments are so pried off these, by means of a knife, that the tissue of the marrow remains unhurt. Cylindrical pieces of marrow are thus obtained which are cut at the ends with a knife or razor to remove the bone sawdust earlier made. Frog tissue was obtained in the same way. Practice renders the process very rapid and free from injury to tissue. The following fixatives were used: 1. Nikiforow's modification of Foa's liquid—equal parts of a five per cent. solution of potassium dichromate and a saturated solution of sublimate in a .6 per cent. salt solution. 2. Osmic mixture for fat; with warm-blooded animals, Hermann's stronger solution; with cold-blooded, either solution, but preferably the weaker. Preparations were left in the liquids for 14–16 hours and then passed through the alcohols (45, 60, 80, 95 per cent.) to absolute in the dark. From 60 per cent. upwards enough iodine is used to make the liquid tea-colored. The whole alcohol treatment lasts three days, of which absolute alcohol requires only 12 hours. The preparation 2–3 hours in Flemming's mixture, in Hermann's 8–10 days, then 24 hours in running water followed by increasing alcohols (as above) for 5–6 days. All preparations are put in a mixture of 3 parts absolute alcohol and 1 part chloroform, then into half and half of each, then in absolute 1 part and chloroform 3 parts, finally into pure chloroform. The whole procedure is conducted in the dark and 12 hours are allowed for each mixture. The next liquid is chloroform saturated with paraffin at the ordinary temperature (12 hours), then chloroform saturate at 25°–27° (24 hours); then 32° saturate (12 hours); lastly pure paraffin (45°–46° melting point) for 2–3 hours. Imbedding takes place either in this or in paraffin of 52° melting point. Microtome sections 5 $\mu$  in thickness are fastened by the glycerine albumen process.

**STAINS**—Hermann and Flemming are stained in the usual 1 per cent. alcoholic safranin solution left on the slide in a moist chamber for 24 hours, washed in water and differentiated in acid alcohol (1:1000). After Nikiforow's mixture hæmatoxylin-eosin is used with Ehrlich-Biondi; also Bordeaux-iron-hæmatoxylin as follows: Preparations remain in the mordant for 5–6 hours, in hæmatoxylin 20–24 hours. The form used was Weigert's or a .5 per cent. aqueous. These solutions can be used repeatedly. Isolation preparations of marrow may be made of marrow previously hardened in Nikiforow's fluid (12–24 hours), washed very thoroughly and stained. Often such tissue may be put into 1 per

cent. osmic acid with a few drops of eosin for 24 hours, and studied in glycerine. Fat is black-brown, nuclei and eosinophile granules red. Difficulty was found in determining the number of eosinophiles in blood from moist preparations. A Thoma-Zeiss counting apparatus was used; the blood is diluted with a fluid which fixes the cell elements and brings out the granules—osmic acid with eosin. The capillary tube is filled to (1) with blood, and then to ( $\frac{1}{2}$ ) with a 1 per cent. osmic acid solution. This acts for 2 minutes. The mixer is filled to the upper line with a fluid composed of 55 parts of distilled water, 45 parts of glycerine and 17 parts of eosin. The blood in the pipette may be directly diluted to the outer mark with the osmic-eosin solution. To about 5 c. c. of a fresh 1 per cent. solution of osmic acid 4–5 drops of the following are added: Distilled water 10 parts, glycerine 10 parts, 1 per cent. aqueous eosin 5 parts. This mixture retains its powers for about one hour. Zappert's counting cell was used. The following objections are pointed out: Fresh osmic mixtures needed every time; granule stain is light; there is difficulty in counting the remaining forms of leucocytes without methyl-violet.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Penard, E. Faune Rhizopodique du Bassin du Léman. Avec nombreuses figures dans le texte. 714 pp. 4to. Henry Kundig, Genève, 1902.

This elaborate and most complete monograph of these cosmopolitan Protozoa contains in an appendix some suggestions from the author's experience with regard to the collection, care of, and preparation of these animals for study.

They are found everywhere in fresh water, especially the surface of the bottom slime and upon aquatic vegetation. *Pelomyxa palustris* is wont to be found in the deeper parts of the slime. Among the water plants *Chara* seems to be avoided by the Rhizopods. For collecting this slime in deep water the author uses a light tin dredge of elongated form. At a distance of two metres in front and at the same distance behind, weights of 300 grams are placed upon the line to hold the apparatus in proper position. If this is drawn slowly and brought to the surface with care some of the superficial ooze can be secured. This is put through a sieve of .6 mm. mesh which removes coarse debris and the larger organisms, while permitting the Rhizopods to pass. Aquaria are stocked with the fine ooze and in a day or two the Rhizopods will be found upon its surface at the bottom of the dish. If the collection is placed in a very tall vessel the animals will creep up upon the sides in great numbers and may be collected apart from the sediment at the bottom by decanting with precaution. In American waters great numbers of Rhizopods will be found in the washings of *Sphagnum* and the silk towing net is also a fruitful source of material.

The author isolates the individual animals from the debris for study or for

staining by floating the cover-glass up from the mount for examination by the addition of more water, and then carefully pushes it to one side until the animal is exposed. The position of the animal is noted, the slide transferred to a dissecting microscope and the adjacent debris removed. The animal is then carefully pushed from its location into an adjacent drop of clean water, the remainder of the debris is removed and the cover-glass replaced if the living animal is to be studied. When permanent preparations are to be made the animal is transferred to a watch glass, the surplus water removed, and when the pseudopodia are fully extended it is flooded with absolute alcohol. It is then lightly stained in borax carmine and mounted in balsam. This brings out the nuclei, which are quite essential for modern classification, but renders indistinct the silicious particles of the shell. The latter must be studied in aqueous media. If mounted in balsam of sufficient consistency the animals may be oriented in any desired position by pushing the cover-glass. This is very desirable in the study of the oral aperture, especially in many of the recently described forms. Whole collections may be treated by this method of killing and staining *en masse* and then assorted as desired. Since Leidy's great work on the Rhizopods no monograph comparable to this of Penard's has appeared. It is indispensable for everyone who desires to work with this group.

C. A. K.

Grave, Caswell. A Method of Rearing Marine Larvæ. Science, N. S. 15: 579-580, 1902.

The principle of balanced aquaria has been applied by the author to the rearing of marine larvæ. The plant life is supplied by diatoms, which are obtained from aquaria stocked with bottom deposits of sand or mud. The diatoms multiply upon the bottom of such aquaria, and may be gathered with a pipette from the surface of the sand. The larvæ are placed in aquaria of fresh sea water and a dozen or more pipettefuls of the diatom ooze are added. This aquarium is then covered and placed near a window where it is well, but indirectly lighted. The diatoms furnish food and oxygen for the larvæ and there is no need for frequent changes of the water. This should be changed occasionally to replenish the salts used by the organisms. Larvæ thrive in such aquaria, and pass successfully through the critical stage of metamorphosis.

C. A. K.

Hickson, S. J., and Wadsworth, J. T. Dendrocometes paradoxus. Part I., Conjugation. Quart. Journ. Micr. Sci. 45: 325-363, pls. 17-18, 1902.

This Suctorian occurs on the gills of *Gammarus pulex* at all seasons of the year, but in greatest abundance in spring and fall. A dozen *Gammarus* placed in a shallow dish with an inch or two of water and some mud and water weed will, within a fortnight, yield a number of conjugating *Dendrocometes*. These parasites are firmly attached to the gills so that they may be manipulated readily through the various processes in the technique of preparation without the danger of loss and the difficulty in orientation which attends the preparation of most *Protozoa*. It is necessary to locate the conjugating pairs upon the gills, which are large enough to be imbedded and cut in any desired plane. Sections were stained in iron-hæmatoxylin and in iron-brazilin. Both *Dendrocometes* and its host occur in American waters.

C. A. K.

## GENERAL PHYSIOLOGY.

- RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoological Laboratory,  
University of Michigan, Ann Arbor, Mich.

Moore, B., and Parker, W. H. The Osmotic Properties of Colloidal Solutions. *Amer. Jour. Physiol.* 7: 261-293, 1902.

The problem which the authors investigated was whether, in a colloidal solution, the large molecule (or "solution

aggregate"), consisting of many chemical molecules in physical combination, behaves like a molecule of crystalloid solution, and exerts osmotic pressure, or is perfectly inert with none of the properties of matter in solution. It has been found by previous workers that such indirect methods of observing osmotic pressure as lowering of freezing point and raising of boiling point in case of colloidal solutions, give results so low as to fall within the limits of experimental error. As a consequence the authors decided to use the method of measuring directly the osmotic pressure by means of an osmometer. To guard against the source of error introduced by the presence of crystalloids in the solution, the following general method was used. The size of the solution aggregate was altered by the action of reagents, which left unaffected the amount of indiffusible or feebly diffusible salts in solution. Since osmotic pressure depends upon the molecular concentration rather than upon the molecular weight, a higher pressure should be obtained when the solution aggregate is broken down and consequently made smaller in size, since there will then be a larger number of such aggregates per unit volume. If the amount of crystalloid is kept the same in both experiments, any observed difference in osmotic pressure must be due to the colloidal substance.

Great difficulty was experienced in obtaining an osmometer in which there

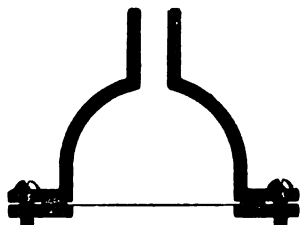


Fig. 1.

was no leakage around the edges of the membrane. An instrument was finally devised by the authors which entirely overcame this difficulty. Their apparatus is shown in section in Fig. 1. It consists essentially of a brass receiver, hemispherical in form, thickly plated with silver, and finally on the inner surface with gold. The purpose of this gold plating is to exclude chemical action of the solution on the metal. The receiver has at its upper pole a

tubular continuation for connection with the manometer, and a broad flange around its circumference, which has a narrow circular groove, let into its under surface. In this groove lies a somewhat thicker rubber band, against the under surface of which the membrane is tightly pressed. The pressure is equally applied by means of four screws (two of which are shown in the section) passing through four equidistant holes in the outer part of the flange of the receiver, and engaging in four similar but threaded holes in a heavy ring of metal of similar dimensions to the flange. In fitting up the instrument for an experiment, the

membrane of parchment paper is cut to the outer diameter of the flange, and the position of the screw holes marked with a pencil; openings opposite to the holes are punched out, and the membrane is wetted and laid evenly over the rubber ring; then the screws are inserted and screwed home, and the receiver filled with the solution. Connection is next made with the manometer by means of a piece of barometer tubing of thick wall and narrow bore. Parchment paper was used as a membrane. The authors found this to be absolutely impermeable to colloids, and in other ways very satisfactory.

The colloid most used in the experiment was sheep's serum. The osmotic pressure of a sample of this was first obtained, and then the serum albumen was converted into alkali albumen by adding a sufficient quantity of ten per cent. caustic soda solution to make a one per cent. solution, and boiling. The same percentage amount of caustic soda solution was then added to the outer fluid, and readings were again taken. A much higher pressure was invariably obtained in the second case, indicating that the size of the solution aggregate in the alkali albumen was smaller than in the serum. In a typical experiment a reading of 22 mm. of mercury was obtained with serum, while with alkali albumen the osmotic pressure was equal to 110 mm. of mercury. Thus the conclusion that a definite osmotic pressure is exerted by colloids in solution is established. The authors are inclined to think that the differences in properties of coagulable proteids may be due to different physical arrangements of chemical molecules to form different aggregates, and that the complexity of the proteid molecule may be much more a physical than a chemical phenomenon.

For an account of the numerous experiments, as well as for the valuable and interesting discussion of the important bearing of the results of this work on general physiological problems, recourse must be had to the original paper.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

Wright and Joslin. Degeneration of the Islands of Langerhans of the Pancreas in Diabetes. The J. of Med. Research, 2: 360-365, 1901.

Schäfer, Diamere, Ssoblew, and Lagenesse have suggested that the islands of Langerhans might play an important part in carbohydrate metabolism. Opie has given this idea strong support. Among fourteen cases of chronic interstitial pancreatitis the islands of Langerhans were found to be the seat of hyaline degeneration in four. In three of these four cases diabetes mellitus had been present, but had been absent in the others. In the fourth case the islands were only slightly affected. Moreover, in two cases of diabetes mellitus Opie found there was no increase in the interstitial connective tissue of the gland, but the epithelial cells of the islands of Langerhans were more or less extensively transformed into hyaline material.

In one the lesion was strictly limited to these structures, but in the other the degeneration had extended more widely. Opie concluded that in pancreatic diabetes the lesion destroys or injures the islands of Langerhans and that where, though the organ is diseased, diabetes is absent the interacinar islands are relatively unaffected.

The pancreas was examined by Wright and Joslin in nine cases of this disease. Hyaline changes in the islands of Langerhans, like those described by Opie, were found in two of these nine cases. In one of them the degeneration was practically confined to these islands. In the other the gland also showed fat necrosis. Wright and Joslin think that lesions of these structures are important factors in the pathology of this disease.

W. R. S.

**Herzog.** Zur Histo-Pathologie des Pankreas beim Diabetes Mellitus. Virchow's Archiv. 168: 83-90, 1902.

The findings of Ssobolew and Opie are here reviewed, and then the work of Weichselbaum and Stangel is considered. These two investigators found an atrophy of the pancreas, especially confined to the islands of Langerhans, in eighteen cases of diabetes mellitus, and could detect no changes in these islands in cases of other pancreatic affections. Herzog's five cases were then published, to be followed by those of Wright and Joslin, above referred to.

In the present article Herzog gives more details concerning his cases. In three of them the pancreas examined was a museum specimen and nothing further was known except that the cases were those of diabetes. In all of them changes were seen in the islands of Langerhans. A decrease in the number of these islands was always noted, ranging from a slight diminution in Case I to their total absence in Case V. In one case only did the islands show distinct hyaline degeneration. No other changes were observed. Following Weichselbaum and Stangel, Herzog thinks the process is essentially a specific atrophy of these islands, of unknown cause. These findings, above given, form added support to the theory that the islands of Langerhans furnish by their internal secretion a sugar-splitting enzyme.

W. R. S.

**Kakels, M. S.** A Contribution to the Study of Primary Sarcoma of the Tail of the Pancreas. Am. J. Med. Sc. 123: 471-480, 1902.

Kakels reports a case of primary sarcoma of the tail of the pancreas. The tumor was not diagnosed correctly, but was thought to be a sarcoma of the kidney. An exploratory laparotomy revealed its true origin. The patient died shortly afterwards, and at autopsy a large, partly hard and partly soft tumor was found in the region of the tail of the pancreas. It was a nodular mass and measured 18 x 9 cm. Microscopically a fine, fibrous, reticulated network was seen, in the meshes of which were a large number of small, round, mononuclear cells whose nuclei took the stain well. There were also a number of larger cells, both polynuclear and mononuclear. Their nuclei also stained deeply. The tumor was quite vascular, and in places had undergone coagulation necrosis. The diagnosis of mixed cell sarcoma was made. Kakels has collected in the literature twenty-one cases of sarcoma of the pancreas. In but three of these was the growth primary in the tail of the gland. His case consequently is the fourth reported.

W. R. S.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, *Wesleyan University.*

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

**Irons, Ernest E.** Neutral Red in the Examination of Water. Abstract of paper read at 3rd Ann. Meet. of Soc. Am. Bact.

In 1898 Bothberger found that *B. coli communis* will reduce neutral red in a culture medium, changing the color from red to a canary yellow, with an accompanying green fluorescence.

Schleffler tested a number of races of *B. coli* and found that all gave the neutral red reaction. In 1901 Savage employed neutral red for the detection of *B. coli communis* in water. He concluded that a positive reaction, obtained with neutral red, while not diagnostic of *B. coli*, yet, in the vast majority of cases, points to the presence of that organism, and that in the case of the fifty waters examined, the margin of error in assuming that *B. coli* was present where a positive neutral red reaction was obtained, was less than five per cent.

The object of the present experiments was to determine further the value of neutral red in the routine examination of water. Following the suggestion of Savage, ordinary bouillon was used, to which was added 1 per cent. of dextrose, and 1 per cent. of a 1 per cent. aqueous solution of neutral red. All cultures were kept at 37°C.

Determinations were made by the dextrose fermentation tube and neutral red methods in exact parallel. Samples of forty-five waters were employed with a number of dilutions of each, such that in the case of each water, *B. coli* was almost always found in the lowest, and rarely in the highest dilution. In this series 285 determinations were made by each method, with 35 per cent. positive results for the fermentation tubes and 47 per cent. positive with neutral red.

H. W. C.

**Cambier, R.** A Contribution Concerning a Method of Investigation for the Typhoid Bacillus. An account given at the sessions of the Academy of Sciences, June 10, and December 23, 1901.

The new method of investigation of the typhoid bacillus, presented to the Academy of Sciences by Dr. Roux, assistant director of the Pasteur Institute at Paris, is extremely simple. It is based upon the ease and rapidity with which this very mobile microbe passes through a porous wall.

Following is the technique employed in the investigation of the bacillus of Eberth, and in its separation from the colon bacillus, according to numerous experiments made by Mr. R. Cambier in collaboration with Mr. A. Girauld. A porcelain filter of the porosity of a Chamberland's "F" should be placed in a large glass tube, closed at one end, and the whole disinfected in a Pasteur oven. The tube should then be filled half full of sterile bouillon, which may be prepared by mixing in the cold and aseptically, 1000 c. c. of a 3 per cent. Defresne peptone solution, sterilized at 115°, 120 c. c. of a sterile 1 per cent. soda solution, and 120 c. c. of a saturated sterile solution of sea salt. Inoculate the interior

of the filter with the liquids which are supposed to contain the typhoid bacillus, and place it in the incubator at  $37^{\circ}$  for from fifteen to forty-eight hours, the length of time depending upon the porosity of the filter. The bouillon surrounding the filter is at first perfectly clear, but soon becomes cloudy, thus demonstrating the passage of the microbe through the pores of the porcelain filter.

When this takes place remove aseptically by means of a slender sterile pipette, a certain quantity of the cloudy bouillon and make the microscopic examination and the tests for the agglutination reaction with the typhoid bacillus; inoculations should also be made upon ordinary bouillon, upon litmus lactose broth, upon milk, lactose gelatin, potato, etc., in order to identify the bacillus. Instead of the alkaline salted bouillon of which we have just spoken, ordinary neutral bouillon may be used; but in that case certain classes of very mobile colon bacilli traverse the porous wall and interfere with the diagnosis. Under these conditions it has been found that the addition to the ordinary neutral bouillon of sodium hydroxid, in increasing amounts, at first furthers the development of the colon bacillus and then hinders it almost immediately. Under the same conditions the development of the bacillus of Eberth continues equally, at a maximum rate for a weak alkalinity; but maintains a perceptibly constant value, equal to the maximum, so long as the amount of sodium hydroxid added to the solution of 3 per cent. Defresne solution does not reach a concentration of more than 1 to 2 grams per liter. If this concentration is exceeded the difficulties which the cultures of the colon bacillus and those of the typhoid present become very considerable, and it is only by the use of extremely alkaline media that mixed cultures of colon and typhoid bacilli may be obtained in which the latter is not destroyed first.

Unfortunately the addition of soda to the bouillon hinders the mobility of the typhoid much more rapidly than it does that of the colon bacillus. Sea salt, added to the bouillon, has a very valuable effect in this method—that of restoring to the typhoid bacillus its original mobility, even after it has been hindered by the soda, and also that of destroying the mobility of any colon bacilli which may have persisted. This special alkaline and salted bouillon possesses the great advantage of allowing the bacillus of Eberth to vegetate under favorable conditions, and to retain its mobility, while hindering the development and mobility of the colon bacillus; in this case alone does the typhoid bacillus traverse the porous wall first.

To sum up: In combining, as has just been said, the action of the soda and the sea salt, a bouillon can be made which, with the help of the cultures on the filters, admits of the separation, beyond a possibility of doubt, of typhoid from a definite colon bacillus. In practice, when the typhoid bacillus is to be separated from water, or from a salt which also contains colon bacilli of unknown nature, it is advisable to inoculate several tubes containing the porous filters, and stocked with alkaline bouillon containing increasing quantities of salt. In order not to alter the concentration of the bouillon, it is wise to introduce into the filter tubes only a few drops of the suspected liquid; or even when it is desirable to make a test upon a considerable volume of the liquid, it is preferable to filter it previously on a porous filter and to use for inoculation only the light film adhering to the surface of this auxiliary filter.

A. GIRAULD.

Tr. by Eleanor L. Lattimore.

## NEWS AND NOTES.

**RICHARD LEACH MADDOX.\***—Richard Leach Maddox was born at Bath in 1816, and died at Southampton May 11, 1902. He was a physician and practiced in many cities of England and southern Europe, but devoted much time to microscopy, photo-micrography and photography.

It is to his experiments in photography that science is most indebted to him, for he was the first to publish a formula by which an emulsion of gelatine and a bromide were used in making dry plates. He gave three reasons for undertaking these experiments: "Firstly, the cost of collodion, with the troublesome manufacture of the cotton; secondly, health more or less affected by its constant use when working, as I was, in my camera, a dressing room, often at a very high temperature in the summer months; and thirdly, dissatisfaction with the dry methods for the photo-micrographic work upon which I was much engaged."

In his original experiments with gelatin, Dr. Maddox pursued the following method: "Thirty grams of Nelson's gelatin were washed in cold water, then left to swell for several hours, when all the water was poured off and the gelatin set in a wide-mouthed bottle, with the addition of four drachms of pure water and two small drops of aqua regia, and then placed in a basin of hot water for solution. Eight grains of bromide of cadmium dissolved in half a drachm of pure water were now added, and the solution stirred gently. Fifteen grains of nitrate of silver were next dissolved in half a drachm of water in a test tube, and the whole taken into the dark room, when the latter was added to the former slowly, stirring the mixture the whole time. This gave a fine milky emulsion, and was left for a little while to settle. A few plates of glass well cleaned were next levelled on a metal plate put over a small lamp; they were, when fully warmed, coated with the emulsion spread to the edges by a glass rod, then returned to their places and left to dry. When dry, the plates had a thin, opalescent appearance, and the deposit of bromide seemed to be very evenly spread in the substance of the substratum. These plates were printed in succession from different negatives, one of which had been taken years since on albumen with ox-gall and diluted phosphoric acid, sensitised in an acid nitrate bath, and developed with pyrogalllic acid, furnishing a beautiful warm brown tint. The exposure varied from the first plate thirty seconds to a minute and a half, as the light was very poor. No vestige of an outline appeared on removal from the printing frame. The plates were dipped in water to wet the surface, and over them was poured a plain solution of pyrogalllic acid, four grains to the ounce of water. Soon a faint but clear image was seen, which gradually intensified up to a certain point, then browned all over; hence the development of the others was stopped at an early stage, the plate washed, and the development continued in fresh pyro, with one drop of a ten-grain solution of nitrate of silver, then re-

\* British Journal of Photography, 49: 2195.

washed and cleared in a solution of hyposulphite of soda. The resulting prints were very delicate in detail, of a color varying between a bistre and olive tint, and after washing dried with a brilliant surface. The color of the prints varied greatly according to exposure."

Although, because of ill health and urgent professional duties, Dr. Maddox was unable to carry his experiments to perfectly successful results, yet, he undoubtedly began the series of experiments which led to the substitution of the gelatin dry plate for the old collodion methods.

Dr. Maddox was also interested in bacteriological research, especially in the study of organisms in the air, for which he used an apparatus of his own invention,—the "aéroconoscope"—a kind of multiple funnel set up as a vane. The wind traversing this instrument deposited the organisms on a thin cover-glass duly prepared for the purpose.

C. W. J.

## QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

### REPLY TO QUESTION No. 24.

Curling of paraffin sections is due to cold. If a microtome is used a lighted lamp brought near the knife will often raise the temperature enough. If not, there is no other remedy than (a) raising the temperature of the laboratory or (b) cutting thinner sections. If sections are being cut by hand, the paraffin may be slightly softened by blowing on it. The same method may be employed when there is difficulty in starting a ribbon on the microtome.

M. A. WILLCOX.

Wellesley College.

## Books Received.

The new edition of Webster's International Dictionary contains a supplement with over 25,000 words. The technical terms used in various branches of science, and particularly the genus and family names in botany and zoölogy, have not hitherto been considered a legitimate feature of a dictionary's contents. Yet the student is in constant need of just such definitions, and it is certainly a source of satisfaction to know that they are here included in a dictionary reasonable in size and reasonable in price.

The definition of scientific terms has been intrusted to the most eminent specialists in the country, such as Dr. Ira Remsen, for chemistry; Prof. G. K. Gilbert, for geology; Prof. Lester F. Ward, for botany, etc. The last subject received an unusual degree of attention, Prof. Ward having been assisted in the preparation of definitions relating to the cryptogams and the flowering plants by Dr. F. H. Knowlton and Mr. Charles Louis Pollard, respectively. Thus every important genus and nearly every family will be found amply characterized, the definitions including statements of the geographic ranges of the plants, their economic uses, etc. The vocabulary of popular plant names is also very full.

# Journal of Applied Microscopy and Laboratory Methods

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## The Biology Laboratories of Morningside College.

Doubtless a large number of your readers are teaching in small colleges which do not possess a special building for science work, and many others are working in so-called "science halls" which were originally designed for other purposes and which are often very poorly adapted to the work now being carried on in them. These teachers read with interest the descriptions of the more extensive laboratories, but, owing to the different conditions, these accounts are sometimes more helpful by way of inspiration than suggestion.

The biology work of the Morningside College is at present being carried on in a set of rooms occupying the south end of Main Hall, a building erected two years ago for general recitation purposes. This building is "T" shaped, the main part fronting toward the east being 140 feet in length, while the east and west dimension is 145 feet.

Rooms with some other exposure might have been secured, but the advantages of the smaller auxilliary rooms led to this choice, and for general biology work in this climate, the advantages of south exposure seem to quite balance its disadvantages. Thus situated, very few days are too dark for microscopical work, even with the higher powers, and on brighter days the direct sunlight can easily be modified by pulling up special inside curtains which were not attached at the time these photographs were taken. I need not dwell on the advantages of direct sunlight for certain kinds of work.

In the shaping of our equipment, several points had to be kept in mind. One question was how to make the best use of rooms not especially constructed for laboratory purposes; it was also desired that the equipment should be readily movable and adaptable to the new quarters when such should be provided; anticipating such removal, the rooms were not to be marred nor disfigured; and lastly, there was the problem of making the best use of a limited appropriation.

The accompanying plan (Fig. 1) shows the general arrangement of these

rooms. The lecture room has elevated seats, and is well lighted from windows at the students' left. The east laboratory (Fig. 2), used for work in morphology, is fitted with wall tables, aquarium and supply tables, students' lockers, and microscope cabinet. The histology and physiology laboratory (Fig. 3), on the west, has a similar equipment with the exception of microscope cabinet. Adjoining is a supply and store room, and also a dark room furnished with running water, for photographic and physiological work. Between the larger laboratories is a smaller private laboratory (Fig. 4) and study, while opening into one of the laboratories is an alcove which is fitted with shelving for the herbarium. An additional room in the basement provides for the storage of rough materials and

FIG. 1.—Floor Plan of Biology Rooms.

supplies not needed for immediate use. Museum space is being provided for elsewhere in the building. The laboratories are equipped with city water, gas, and electric lights, and, in harmony with the general interior of the building, all furniture is of solid oak.

Wall tables were used instead of individual desks, as they are very much cheaper and economize space. These tables are 28 inches wide and 29 inches in height. They are rigidly fastened to the walls, are very strong and free from vibration. The tops are  $1\frac{1}{4}$  inch in thickness and have underneath drawers 24 inches wide and  $3\frac{1}{2}$  inches deep, with 3-inch spaces between drawers laterally.

In institutions of this character, where many students have to use the same laboratory, individual lockers should be provided for safe keeping of instruments, material, note books, etc. Our lockers (Fig. 5) are arranged in banks built

solidly together, but independent of the wall, so that they can be moved bodily without great inconvenience when it is necessary. They could, if the space were needed, be placed out in the hallway. Each locker is 12 x 14 inches, and is 15 inches in depth, and each has a combination lock, the combination being known only by the student who uses it and by the director of the laboratory. The combinations can be changed from time to time as circumstances may demand. This plan avoids all confusion over lost keys and allows the laboratories to be kept open all day so that students who wish to make up back work,

FIG. 2.—Morphology Laboratory.

etc., at irregular hours, can do so even though the one having the laboratory in charge is not present.

Your readers will recognize the microscope cabinet (Fig. 5) as a modification of one previously described in the pages of this journal (Vol. I, p 29). Each receptacle in this cabinet is 10 inches wide and 12 inches high, and is deep enough to hold two microscopes. One row of lockers across the cabinet is displaced by drawers, each having lock, for the storage of cover glasses, extra oculars, micrometers, etc. Two or three of the receptacles have special doors and keyless locks and are thus protected while the outer doors are open. When needed regularly the microscopes are left out on the tables and are covered, when not in use, by cardboard cones. The cabinet affords a safer place for keeping them during vacation, or when not needed daily.

The movable laboratory tables are each  $8\frac{1}{2} \times 8$  feet, and are provided with drawers underneath. These are used as microtome tables, aquarium and supply tables, etc., or are used as laboratory tables by the students when the wall tables are all occupied.

For general class use, we provide microscopes fitted with two oculars and with two-thirds and one-sixth inch objectives. Two or three better instruments are usually left out to be used as demonstration microscopes. For classes in histology and for finer work in morphology, a better stand is used which is fitted

FIG. 3.—Physiology and Histology Laboratory.

with special illuminating apparatus. The major students in biology have the advantage of microscopes equipped with oil immersion lens and camera lucida.

The histology laboratory has two water baths, an improved Minot rotary microtome, a sliding microtome, and a hand microtome. The department possesses a long focus  $5 \times 7$  camera. No mention need be made of the numerous lesser pieces of apparatus, etc., which are taken for granted in any working laboratory.

Each student is provided with a compound microscope (during his laboratory periods), a dissecting microscope, a case of dissecting instruments, reagent bottles, and use of a locker. All apparatus is checked against the user when it is exclusively for his use.

FIG. 4.—Private Laboratory and Study.

FIG. 5.—Microscope Cabinet and One Set of Students' Lockers.

With this equipment we have been able to conduct Biology courses which, during the past term, had a total class enrollment of over one hundred students, allowing each student the exclusive use of a microscope during his laboratory hours. This has been made possible, however, only by breaking up the classes into numerous laboratory divisions. With the erection of our new science building, everything can be moved from our present laboratories in a couple of hours, leaving only a few nail holes and three gas plugs to tell of former tenants.

Morningside College. ROBERT B. WYLIE.

### Hints on Collecting Land and Fresh-Water Mollusca.

The collector should always bear in mind:

*First.* That a dead shell is better than none at all.

*Second.* That dead shells should not be taken, if live ones can be had and that careful search will usually discover them wherever their "bones" are found.

*Third.* That all the species are extremely variable in their abundance from year to year, so it is a safe rule "when you're getting, to get a plenty."

#### COLLECTING APPARATUS.

For land shells, a "Ferriss" hoe is indispensable. This is made by getting a small, light-handed garden hoe and having the blade cut down at a machine shop. It should be about three inches wide on top and taper to a sharp point. Then cut off the handle so that it will be as long as a cane. This makes a most convenient tool for turning over logs and breaking up rotten wood, digging around stumps and among the dead leaves, and in a snake country is very effective for killing rattlesnakes. A pair of fine curved-pointed collecting forceps is also necessary for picking up the small species. Small glass or wooden bottles should be carried, as the small species are apt to get lost in the dirt and slime, if put into the same receptacle as the larger ones. It is better not to put the small species in alcohol as they are collected, as they are then killed at once with the animal more or less extended. If put in a dry bottle and left a few hours they will withdraw into their shells, leaving the aperture clear and fit for examination. This is especially necessary with the *Pupidae*, where the arrangement of the apertural teeth is a specific characteristic.

For the larger species tin cases of a convenient size to slip readily into the coat pockets are most convenient. Where the larger *Helices* are so abundant as to make it annoying to have to take the cover off continually, it will be found convenient to have a square hole cut in the cover large enough to pass a snail through. Then stretch a piece of thin rubber, such as a piece of bicycle-tire lining, across the top of the can, and put the cover in place so as to keep it stretched tight. A slit in the rubber corresponding to the hole in the can will enable one to slip the snails into the can, and the elasticity of the rubber will keep them from getting out again. A few leaves should be put into the cans before starting out to prevent the shells from being injured by rolling around

inside. As the cans become full, the leaves can be removed. Several boxes and a number of vials should always be carried, so that specimens from different localities may be kept separate.

For the fluviatile species it is necessary to have a dipper and, if possible, a small dredge. The dipper is made from an ordinary tin one, by removing the bottom and substituting one of fine wire cloth. By removing the end of the handle, the dipper can be slipped on to the end of a cane or pole when in use. This is useful not only for reaching the larger specimens from the shore or boat, but especially for sifting the mud and sand from the bottom, where a multitude of small species live, which otherwise would not be found. It will be found more convenient to empty the contents of the dipper, when thoroughly washed out, into a pail and carry the whole mass home before undertaking to pick out the shells. If attempted in the field, many of the smaller and more desirable things are apt to be overlooked. By spreading the mass out in the sun for a short time it will become dry and friable so that the shells can be easily separated and picked out. An ordinary reading glass is very useful for the detection of the more minute forms in sorting over such material.

Many desirable species live in water too deep for the convenient use of the dipper, and for these it is necessary to have a small dredge. One with an aperture of 9 x 6 inches is as large as can satisfactorily be used by a single person in a row boat.

Care must be taken to keep the more fragile species separate from the heavier ones, otherwise they are apt to be damaged in carrying the can about.

#### WHERE TO COLLECT.

Everywhere. The land species love dampness and darkness. They are to be looked for under logs, bark, and leaves in suitable localities. Many species bury themselves in rotten logs, and these should be broken up with the hoe. The accumulation of dead leaves around fallen trees is a favorite habitat and should also be carefully and slowly gone over with fingers and hoe. The thick grass and dense thickets along the margin of ditches and streams will usually reward a careful examination. Southern and western exposures being dryer, are not so fruitful as eastern and northern hillsides and shady ravines. Coniferous forests are usually quite barren of molluscan life. An open hardwood forest in a limestone region is the ideal hunting ground. Nearly every permanent body of water has its mollusks, varying according to its character. Some species are found only in rapid flowing water, and others only in ponds and still water. Ditches and other stagnant waters are usually good collecting ground for *Pisidia* and other small species. The low places in the woods, which dry up in the summer time, have a number of species that are not found elsewhere, and which bury themselves in the mud when the water disappears. Sand banks in rivers and lakes are the favorite resort of many of the smaller species. The under side of the lily pads should be scrutinized, while the *Ancylus* should be looked for on stones and dead clam shells.



## CLEANING AND PREPARATION OF SPECIMENS.

The larger *Helices* should not be put into alcohol unless desired for anatomical purposes, as it is almost impossible to remove the animal after it has become hardened. They should be boiled as soon as possible. Dead snails have a fragrance peculiar to themselves, and seldom make good specimens. The water should be boiling, not simply hot. Species of about the same size should be boiled together in order that the operation may be successful. A small wire strainer with a long handle is very convenient for holding the snails while boiling. If dropped directly into the water, there is apt to be trouble in fishing them out and they are likely to be boiled too much. The time varies according to the size and the species, some requiring more time than others. If not boiled enough, the muscular attachment to the shell will not be loosened, and the animal will not "pull" at all. If boiled too long, it is apt to break in two and give a good deal of trouble before extraction. The time required varies from ten seconds for a species of the size of *Polygyra monodon* to sixty seconds for *P. albolabris*. It is well to experiment a little at first with a specimen or two of each kind until the proper time is found. Only a few should be boiled at a time, as they "pull" easier while warm. When boiled, the animal should be slowly and carefully pulled out. Too much haste is apt to cause the animal to break apart, leaving the apical whorls still in the shell. The curved points of the collecting forceps are convenient for extracting the animals and hooks of various sizes can be made from safety pins. By tying these on to small wooden handles very effective instruments can be made. A small, fine-pointed dental syringe is indispensable for this work. If the animal cannot be started with the hook, or if it breaks in two, a jet of water from the syringe will usually solve the difficulty. When the apical whorls are left in the shell, they can sometimes be started by holding the shell carefully in hand so as not to crush it, and then striking the fist sharply on the thigh of the operator. In case very desirable specimens get into this predicament, putting them in alcohol for twenty-four hours will contract the remnant of the animal sufficiently to enable the successful use of the syringe. Many of the species have the aperture so obstructed with teeth, that it is difficult to extract the animal with the hook. In such case a vigorous use of the syringe will force enough of the body out of the shell to enable the hook to be used. Frequently the whole animal can be forced from the shell in this way, especially in the smaller forms. Patience and perseverance will clean nearly every specimen. When the animal is completely extracted, the interior should be thoroughly washed out with the syringe. A small piece of sponge on the end of a fine copper wire, which can be bent in any direction, is very useful for removing the mucous, which is apt to adhere to the interior of the shell. This should always be carefully attended to, as it will greatly disfigure the specimen when dried. The exterior should then be thoroughly scrubbed with a soft tooth or nail-brush. When perfectly clean, inside and out, the water should be carefully emptied out and the shell put aside in the air, but not in the sun, to dry. No oil or acid should be used on any of the land shells. It is not desirable to attempt to clean the small species by removing the animals.

By keeping them for a short time in a dry place, the animal will retire far within the shell. Then they should be put into 25 per cent. alcohol for a day or two. If to be left longer in the alcohol, the strength should be increased. Twenty-four hours, however, in the alcohol is all that is necessary. Then they can be dried in the air without leaving any offensive odor. Either before or after drying they can be cleaned by putting them in a bottle with some fine, clean sand and shaking them together until all the dirt has been removed by the sand.

Specimens of land snails desired for anatomical purposes should be drowned before being put into the alcohol or formaldehyde, otherwise they retire as far as possible within the shell and are more difficult to dissect in this contracted condition. By drowning, they die fully extended and can then be put into the preservative. It takes about a day to kill a snail in this way. The alcohol at first should be greatly diluted, not stronger than 25 per cent., after a day or two the specimens should be removed to 50 per cent. alcohol, and later to the undiluted. Formaldehyde, 2 per cent. dilution, is an admirable preservative for material of this kind.

With the exception of the larger species of *Planorbis*, which are more easily cleaned by boiling, it is practically immaterial whether the fluviatile univalves are boiled or put directly into diluted alcohol. In either case there is no difficulty in extracting the animals. The minute species are treated the same as the small land shells. In the operculate species, it is desirable to retain the opercula of, at least, part of the specimens. While it adds to the labor, it increases the value of the specimen, if it is always done. These are easily removed from the animal and, after being cleaned, should be put inside the shell and the aperture plugged with cotton. All the foreign matter both inside and outside of the shell should be removed by thorough washing. All the water species are apt to be more or less incrustated with deposits of lime or oxide of iron. These can be removed by immersing them in oxalic acid. Care should be taken not to prolong the operation, or the texture of the shell may be injured. Elbow grease is the most effective agent for making good specimens. When that fails, use the acid. The *Ancylus* are always more or less coated in this way, and can easily be cleaned by floating them for a few seconds on the acid, upside down, and then gently brushing them off with a soft brush while held on the tip of the finger.

The larger bivalves should be well washed and, if necessary, scraped off with the knife as soon as taken, care being taken not to injure the epidermis.

They can be boiled, if desired, when the shells will open and the animals easily removed. But as a rule it is more convenient to cut the muscles, which hold the valves together, with a thin bladed knife and scrape the animal out. Care should be taken not to break the edge of the fragile species when inserting the knife. All traces of animal matter should be removed, and after a thorough washing the valves tied together with a string until thoroughly dried. Never use colored twine for this purpose, as it is apt to stain the shells. Any surface incrustation can be removed either with oxalic or muriatic acid. The latter is more convenient for the larger species, and can be applied with a small brush. It does not bite the fingers, so that it can be used freely. Care, however, must

be exercised in using it and the specimens frequently washed, lest damage be done to the shell. The smaller bivalves, the *Sphæria* and *Pisidia*, are best treated by putting into diluted alcohol for a day or two and then drying them. If left too long the shells are apt to open, which interferes with the looks of the specimens.

The larger species of *Sphærium* are better with the animal removed. This can be done after boiling or a few days in alcohol. As these are usually too small to be easily tied together to keep the valves from gaping, each specimen, while the hinge is flexible, should be closely wrapped up in a small piece of tissue paper until completely dry.

A good collection is characterized by two essentials:

*First.* The careful selection and preparation of the specimens themselves.

*Second.* Absolute accuracy in the matter of the localities from which they come.

There is little excuse for having poor and ill-cleaned specimens. There is none at all for failure to keep accurate records of the collector's field work. A drawer of a common species, such as *Polygyra albolobris*, without any indication from where they came, even if well cleaned, would be absolutely without value. Such a drawer of any of our species from fifty or one hundred different localities, definitely indicated, would be a valuable contribution to the conchology of any state.

Specific names can be supplied or corrected any time, but a mistaken or erroneous locality cannot ever be corrected. The collector therefore should be careful never to trust to memory for facts of this kind.

Both in collecting and cleaning, the specimens from each locality should be kept carefully separated and labeled. Too much importance cannot be given to this point. The study of the geographical distribution of the mollusca is one of the most important branches of conchological work, and this, to be of any value, must be based on absolutely accurate work on the part of the collector.

#### ARRANGEMENT OF THE COLLECTION.

The manner of casing and arranging the collection is largely one of individual preference. A catalogue, however, is essential, and it is better to begin systematically in this particular and thus avoid the necessity of doing it all over again, when the collection begins to assume considerable size. There should be a serial catalogue and a card catalogue. Each addition to the collection should be numbered as soon as received and entered in the serial catalogue, which should be a book ruled in as many columns as the collector desires. A convenient form is as follows:

Serial No.	No. of Spec.	NAME.	From whom Received.	Locality.	Remarks.
1	5	<i>Polygyra albolobris</i> (Say).	John Jones	Ann Arbor, Mich.	

The card catalogue is convenient in a small collection. It becomes absolutely necessary in a large one.

The cards should be of uniform size for convenience in handling. If it is desired to have a card for every entry, they can be smaller than where it is desirable for economy of space to have as many entries as possible on one card. In the latter case a convenient size is that of the ordinary library card, which can be ruled to hold twenty entries. The following is a form of such a card:

<i>Albolabris (Say).</i>	<i>Polygyra</i>
No. 1. Ann Arbor, Mich.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.
No.	No.

The name of the species is written on the top, and the number of each entry of that species and the locality is entered below. Such a card as this enables the collector to see at a glance not only whether any given species is represented in his collection, but also from what localities, and saves a large amount of time which would otherwise be spent in turning over the leaves of a serial catalogue.

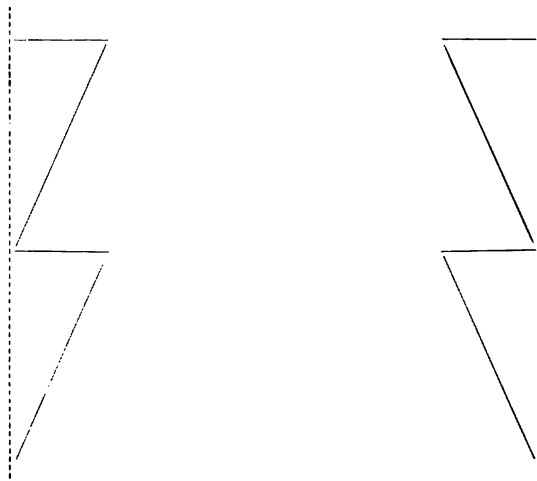
The cards can be kept in drawers or boxes of proper size and can be arranged alphabetically under the different genera and families. Guide cards slightly higher than the ordinary card, indicating the genera, can be inserted in their proper places.

In collections intended for public exhibition, it is usually necessary to have the specimens mounted on cards or blocks. But in private collections such an arrangement is a mistake. Not only on account of the greater room required for the collection, but particularly because it prevents the handling of the specimens for purposes of study.

Specimens under an inch in diameter are most conveniently kept in glass vials. These can be obtained from any wholesale druggist. They should be without a neck and of standard sizes. The length will depend upon the standard size of the tray adopted. For my own collection I use four sizes,  $\frac{1}{4}$ ,  $\frac{1}{2}$ ,  $\frac{5}{8}$ , and  $\frac{3}{4}$  inches in diameter. As these vials are rather fragile, the pressure of the cork is apt to break them. The cork should therefore be softened by rolling or crushing. A pair of plumber's burner-plyers is useful for this purpose. The

serial number should be put on the cork or on a small piece of paper inside. Specimens too large for the vials should have the number on the shell in ink. Then, if a drawer happens to be overturned, the specimens can be sorted out again without difficulty.

When numbered, the vials and specimens should be placed in trays. For these a standard size should be adopted, so that they will conveniently fit into the drawers of the cabinet. In the National Museum at Washington, the unit is 1 x 2 inches, and the larger sizes are all multiples of that unit. In the Academy of Natural Sciences at Philadelphia the unit is 1 x 3 inches. In either case a convenient intermediate size is  $1\frac{1}{2}$  x 2 or 3, as the case may be. There is one advantage in the use of the larger unit where space is a serious question. For the small species the vials may be made only  $1\frac{1}{4}$  inches in length, and two vials can be put into one tray, thus doubling the capacity of the drawer. The trays should be of the same depth. One-half inch is sufficient for most of the univalve species. For the larger species and the *Unionidae*, requiring trays of good size,  $\frac{3}{4}$  inch is better. These trays can be had of any paper box manufacturer, or can be easily cut out of cardboard by the collector, the corners being fastened together by strips of gummed paper. The character of the cases for a collection is determined by the means and inclination of the collector. Any case of shallow drawers will do. If, however, cases are to be made, they should be made of a standard size with interchangeable drawers. The standard drawer should be one inch in depth on the outside. The length and width may be of any size, provided they are always the same; 19 x 19 inches inside measurement is a convenient size for a case with two rows of drawers. Other drawers may be 2 inches or 3 inches in depth, but comparatively few of the latter size will be required. The cases may be of any height desired, according to the number of drawers needed. The method of hanging the drawers is as follows: On the inside of the cases are fastened a series of horizontal cleats one inch in depth and  $\frac{3}{8}$  in width at the broad end, extending from the top to the bottom. The following diagram will show the arrangement:



In the one-inch drawers the entire outer edge is beveled to fit the angle of the cleat, and the drawer runs on the projecting edge. In the other drawers a projecting rail is set into the side one inch from the top of the drawer, which serves the same purpose. In this way the drawers fit close to each other and no space is lost.

The doors of the case may be of glass or paneled with wood. A thin strip of rubber set just inside the jamb, so that the door will press tightly against it when closed, serves as an efficient dust protector. It is a great nuisance, as well as a constant source of expense, to attempt to have a glass cover for each individual drawer. Cases made as above are practically dust proof.

Each tray should have a neat label giving the serial number, the name and the locality of the specimens it contains. A box, bottom-side up, can be used for separating the genera and species in each drawer. Small labels of convenient size for use in them can be had, already gummed, at any bookseller's.

Detroit, Mich.

BRYANT WALKER.

## The Kitchen of the Twentieth Century.

This *new* kitchen to which we are rapidly approaching shall justly be termed the laboratory of the household. It will be equipped with carefully made stoves with applied fuels and thermometers, doing away entirely with guess work ; such a kitchen will not under any circumstances contain either a wood or coal stove. Labor saving contrivances will be kept at hand and constantly used ; a dish washing machine will have its own special table at a convenient and proper height. Cooking tables will be covered with zinc or some form of tile not perishable, that will entirely remove the necessity of scrubbing. The walls with round corners will be hard, and composed of material easily washed and made anti-septic. The sink will be ceramic, with either slate or marble drain boards, with a large marble slab at the back, and will be entirely free from any form of wood work. All pipes will be in view, so that they can be readily cleaned ; traps made to open easily, and the cook provided with necessary tools for the operation. There will be a small closet in the kitchen, holding chemicals for cleaning spigots, boilers, and the necessary metal utensils. These, however, will be few, as all utensils will be made of materials that require washing only ; scouring and scrubbing will be things of the past. Stains or grease spots will be neutralized, chemically treated, not scrubbed.

A microscope will have the most convenient and lightest place in the kitchen, and will be in daily use for the detection of adulterations ; the examination of moulds and other products of infection. By its aid the housewife will learn the different yeasts, moulds, and bacteria, how to select the first and avoid the others. And then, too, by the aid of the microscope, she will be enabled to save at least a fifth of her daily allowance and provide her family with much more wholesome foods. For instance, when she buys arrowroot, it will be examined at once to see whether she has received arrowroot, for which a high price has been paid, or

its usual adulterant, potato starch. The microscope will show at once whether the expensive egg powder purchased is egg or simply cornstarch, colored.

The housekeepers' class, under my instruction, purchased a package of "pudding" mixture for fifteen cents. Each member of the class, after one lesson on the microscope, from which they transferred their observations to the blackboard that they might be indelibly engraved upon their sights, recognized at once that they had paid fifteen cents for a package of corn starch, which, purchased under the name of corn starch, would have cost five cents. A similar experiment was given to mustard; true mustard was examined carefully under the microscope, then some of the compounds sold under the name of mustard examined; wheat starch was immediately found and determined. The microscope is also useful in the examination of tea. The colored teas, those having been used and re-colored, can be quickly identified; this not only saves the pocketbook, but the health as well.

Each starch grain has its own individuality and they soon become familiar. The granules of potato starch look like tiny oyster shells, arrowroot, similar but much smaller; while the starch of the canna (*tous les mois*) is round, with concentric markings; corn, like a folded up dumpling with hilum in center. Put a little of the outside of the cheap dried fruits under the microscope and then tell me if you care to eat them.

Take the æsthetic side of cooking, it is rather comfortable to know that one has minutely examined the fruit products in daily use. The kitchen becomes a laboratory over which presides an intelligent woman. The whole household is affected by the change and we are really living.

The housekeeper of the twentieth century must be an educated, scientific woman; then she will find the kitchen the most interesting room in the house. Meals will be quickly gotten and easily served; not over-done or burned, and will contain all the elements necessary to body building. Bills will be lessened and the health of the family much better. Such a one will know the true condition of the fiber of meat. Wash a small piece and put it under the microscope and observe how the fibers are bound or tied together by the connective tissue. Study the texture of the different cuts of meat and adapt to each the suitable method of cooking. Examine the fibre of fish, compare it with lobster or beef, and you will see why they require a longer time for digestion. Slip into the kitchen and put bits of algæ or lichen under the microscope and observe their marvelous structure. Take up the æsthetic side of the household; study and learn from these simple growths how the more complex ones have been formed, look up the decayed portion of fruits, and this will assist in the sterilization and preservation of your canned fruits and vegetables. In a short time housekeeping is relieved of all its terrors; one is capable of sterilizing all the necessary vegetables and fruits, which gives a comfortable table during the winter months at very little cost. If one understands the word *cleanliness*, all canning is easily done.

The time is coming, in fact it is almost here, when the kitchen will be interesting to educated women, and the term "*cook*" will not be applied to the ordinary woman who is really a scullery maid, but to women of intelligence.

Children will not then have spindle legs and weak digestion, but will be robust, well-formed, and healthy.

Cooking, then, will not be drudgery, but a pleasant, useful, honored profession. Let each housewife aid in bringing about these conditions by carefully furnishing and overseeing her own kitchen. This does not mean that she must cook, but she must be the *officer* in command. SARAH TYSON RORER.

Phila. Cooking School.

### A Pocket Magnifier and a Pocket Microscope.

Of the various sciences in which it is desirable nowadays to conduct work in the field as well as in the laboratory, geology seems to struggle under the heaviest load of equipment. At best the geological kit is seldom small or light, at worst it is an impediment.

In all departments the appliances which seem "indispensable" are often times sufficient in number to seriously impede progress. But it is even worse in the case of the geologist, especially if acting in the capacity of state geologist. In that event the people, feeling as they do a kindly though trying sort of proprietorship in the man whose work is maintained at public expense, ply him with every manner of question to be conjured out of the scientific domain. So the dispenser of general information must have his weights and measures with him as a matter of self-protection. In all justice, however, it must be stated that these people are reasonable, readily satisfied, and appreciative to the last degree, especially if answered at the time. They dislike a decision which is waived or in any way postponed to be "reported on later."

4

1

3

PLATE 1.—A Pocket Microscope and a Pocket Magnifier.  
FIG. 1—Pocket, or Field Microscope, open. FIG. 2—Same closed. FIG. 3.—Lens case for comparison as to size.  
FIG. 4.—A watch-shaped pocket lens with Hasting triplets of 5, 10, and 20 diameters, together with a compass.

A simple test with the magnet, streak stone, or blowpipe, or a demonstration under the field microscope readily dispels their doubts. In addition to the geological hammer and pick, one must carry a camera and tripod, books, note books, maps, clinometer, barometer, thermometer, altimeter, compass, blowpipe, and other things, on ordinary occasions, with a microscope for certain occasions. Add to all these the clothing necessary for the trip, and the hand luggage becomes sufficiently burdensome to render compact apparatus very desirable, if not actually necessary. Finding a good pocket magnifier a necessity, and a pocket microscope an important adjunct to the traveling kit, and finding none of these things in the market, drawings were prepared from which the Bausch & Lomb Optical Company made the two glasses described and figured here.

Next to his penknife and watch, the geologist uses his pocket magnifier most, hence it ought to be first-class, and as well made and as good optically as means will allow, in order that it may be lived with somewhat as one lives with his watch, which is chained to him. The price of so inseparable and indispensable a pocket companion is not to be considered so much as its quality, seeing it is likely to be with one for a lifetime, and ought to be the very best from the outset.

The idea was to design a pocket magnifier which should fit in the vest pocket, or in the fob like a small flat watch, free from angles and corners. The size of the one shown here may be still further reduced by dispensing with the compass, although it is a great convenience. The expense may be lowered by substituting ordinary lenses for the Hasting triplets and by mounting in rubber instead of metal, if need be. One is less aware of the presence in his pocket of such a watch-like magnifier, than he is of the smallest ordinary pocket lens with its edges and rings.

Three magnifications are obtainable, namely: 5, 10, and 20 diameters. The writer finds this particular form strong, compact, optically the very best, and mechanically just to his liking. It is chained fast, and is never missing or out of place, and altogether may be pronounced a convenient and desirable magnifier.

#### A POCKET MICROSCOPE.

A pocket microscope, or field microscope, though less easily designed, is nevertheless a possibility, and furthermore it may be small enough to carry in the vest pocket, the entire size being scarcely larger than an objective case. Of course such a microscope lacks the high magnifying power and the conveniences of the laboratory stand, but it makes up for this in a compactness such that it occupies but a mere corner in one's traveling bag. Besides, in field work, high power and convenience are not essential, since the objects to be determined are generally large, such as volcanic ash, chalk, clay, silt, sand, and other relatively coarse material, though that as fine as diatomaceous earth is readily resolved. In many localities these materials are not easily distinguished by the eye, but are instantly detected by this pocket microscope. The following magnifications are obtained, namely, 100, 60, 40, 30, 20, and 15 diameters, which serve the purpose. Besides, the optical parts may be adapted to the dissecting stand, and can thus serve a double purpose.

One of the best features of this little field microscope is the conviction which

it brings to the minds of those who see it resolve a "valuable resource" into common silt, or something of the sort, and thus satisfy the beholder without argument, explanation, or correspondence that further expense and labor on the development of that particular resource is futile. Though less useful than the pocket magnifier, it is nevertheless an important instrument in the kit of the field geologist.

ERWIN HINCKLEY BARBOUR.

University of Nebraska.

### An Easily Constructed Thermostat.

Those who attempt to regulate the temperature of water-baths, incubators, etc., which require a rather low degree of heat, nearly always experience considerable difficulty if the ordinary Reichert thermostat is used. The mercury reservoir in this form of instrument is too small and the pinhole escape for the gas from the inlet pipe far too large to admit of the rapid and accurate regulation necessary for many kinds of work.

After struggling with this problem for some time, the writer devised a very efficient and inexpensive form of instrument which may be easily constructed by anyone of average ability. The principle is, of course, the familiar one, but the method of construction is new.

The body of the instrument is an ordinary sideneck test-tube. One should be selected of rather thick glass and as large as can be taken by the tubulature of the water bath.

From a one-hole soft rubber stopper which fits the test-tube snugly, cut off about 10 mm. from the lower end and into it fit a piece of tubing of about 4 mm. internal diameter and about 75 mm. length (*e*, Fig. 1). Carefully push the plug thus formed down into the test-tube until it reaches a point about 40 mm. from the bottom (*f*, Fig. 1). In case the stopper does not fit closely enough, a small quantity of melted paraffine poured in will remedy the fault.

The inlet tube (*a*, Fig. 1) is more difficult to make, and, unless one is expert, some practice must precede the making of a perfect one. The lower end of the tube should end in a long, straight, slender point, and the pinhole by-pass should be as small as it can possibly be made. It is best to begin by drawing out the tube to make the point. Select a tube about 5 mm. diameter or less and, at a convenient distance (120 mm.) from one end, heat it strongly in the flame of an ordinary Bunsen burner. When it is thoroughly soft, remove it from the flame, and at the same instant draw it out into a slender thread. A few attempts will probably result in a satisfactory tube and one can now pass to the more difficult operation of blowing the pinhole.

FIG. 1.—*a*, Inlet tube for gas. *b*, Rubber stopper. *c*, Pinhole by-pass. *d*, Outlet tube for gas. *e*, Tube in which the mercury rises and falls. *f*, Rubber stopper. *g*, Cistern filled with metallic mercury.

Close the slender end of the tube by holding the tip for an instant in the flame. Select a point near the base of the drawn out portion of the tube and heat it in as small an area as possible by directing against it the tip of a blow-pipe flame. When the spot has become white hot blow strongly into the tube and force the side out into a small bulb, but do not blow hard enough to burst it or form a large bulb. Let this cool a minute or two, then blow air into the tube and hold it imprisoned there by placing the finger tightly over the open end. Direct the finest possible flame against one spot in the bulb and the imprisoned, compressed air will blow out through a very minute smooth hole. The bulb and the hole, if too large, may be reduced in size by heating the tube in the bunsen or blowpipe flame, but care must be taken that the sides do not flow together and close the opening completely. If the hole is much too large it may still be made to answer by pasting over it a bit of paper in which a hole is pricked with a very fine needle.

Fit the inlet tube in the hole of the remaining piece of the rubber stopper, break off the closed tip so that the gas may escape freely from it, fill the lower part of the test-tube and about half of the tube *c* with metallic mercury, and insert the slender end of the inlet tube into tube *c* and push the upper rubber stopper into position. Carefully raise the inlet tube until its lower end is about 5 mm. above the surface of the mercury in tube *c*.

The apparatus is now ready for connection with the gas supply and bunsen burner. The desired temperature having been reached, any further increase is prevented by sliding the inlet tube down until its tip is closed by the mercury.

An improvement on this instrument has been occasionally introduced in the form of a second tube extending through both stoppers and well into the mercury. An iron screw or good sized iron wire can be raised or lowered into the mercury, thus causing it to rise or fall in tube *c*, or an additional supply of mercury can be introduced to bring that in tube *c* to the proper level.

Howard University.

W. P. HAY.

## Nitrogen Fixing Bacteria.<sup>1</sup>

In the creation of plant food in the soil, bacteria play a very important part. Of the many different organisms concerned with the formation of this plant food those that gradually bring about the changes in the condition of the nitrogen atoms are the most interesting as well as the most important. Just how the changes occur that result in the transformation of the nitrogen of the complex proteid molecule into that of nitrites and nitrates is not entirely clear. There is also still much darkness surrounding the movement of nitrogen in the soil, its change from the uncombined state in the atmosphere to the combined forms in the soil. We do know that bacteria, in one way or another, are directly concerned with the fixation of nitrogen. Organisms living in symbiosis with leguminous plants, and others living independently in the soil add to it great quantities of nitrogen. They are the agents that help to maintain, and even increase, the store of combined nitrogen in the soil.

There are probably a half dozen organisms known to-day that have been demonstrated to possess the power of fixing atmospheric nitrogen independently of legumes. It appears, however, that such fixation takes place only in soils poor in available nitrogen. Of these bacteria, there is one at least that exerts a denitrifying effect in the presence of nitrates, while it makes use of free nitrogen in nitrogen-poor media. There seems to be some relation between denitrification and fixation; just what this relation is has not yet been established.

<sup>1</sup> Abstract of paper read before the New Jersey State Microscopical Society by Mr. J. G. Lipman.

### A Simple Fixing Oven for Blood Preparations.

In order to obtain satisfactory and uniform results in the staining of blood spreads, it is necessary that the specimen be properly fixed before staining, as the reaction to stains varies with the method and degree of fixation. Some stains require special methods of fixation, for which no special apparatus is necessary, but for general work fixation by heat is preferable and, with some stains, necessary. Ehrlich's triacid mixture, or some modification of the same, is without doubt the most generally useful blood stain now in use, and requires fixation of the blood film by heat for the best results.



FIG. 1.—A Simple Fixing Oven for Blood Preparations.

For heat fixation the copper plate or bar is far from being satisfactory; some spreads will be overheated, some underheated, while a few will be subjected to the right temperature. It is difficult to determine the proper length of time to apply the heat, and, at best, but a few specimens can be fixed at a time. By this method it is very difficult to obtain reliable and uniform results.

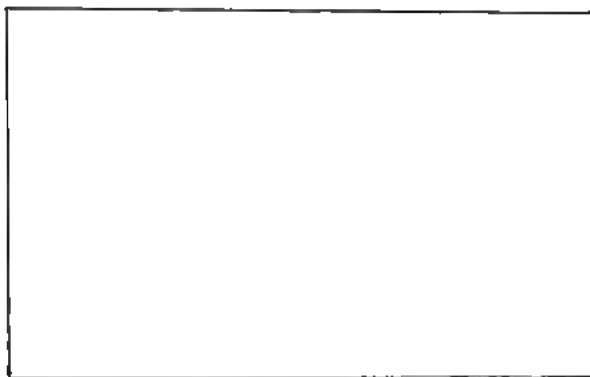


FIG. 2.—The Oven opened; showing Tray for Cover-glasses.

The sterilizing oven gives perfect results, but is comparatively expensive and for such work is slow and not economical. Very few general practitioners possess such an oven and for those who do not do general laboratory work it is an unnecessary adjunct.

Failing to find any special apparatus listed for heat fixation of blood spreads, I devised a small oven for the purpose and had it made by a tinsmith. The apparatus is so convenient and satisfactory that it occurred to me that it might be worth while to send a description of it to the JOURNAL for the convenience of others who do blood work.

The oven is made of sheet copper and consists of a rectangular box containing a tray for cover glasses and a cover that lifts off entire. The whole is supported by a rectangular stand made of  $\frac{3}{8}$ -inch strap iron. The tray is supported by four clips hung from the upper edge of the sides of the rectangular box and lifts out by means of loops of copper wire attached to the ends. The cover fits loosely and has a wooden knob bolted to the center and has a tubulation at one end for thermometer.

The dimensions, which may be varied to suit requirements, are as follows: length, 9 inches; width, 4 inches; depth, 2 inches. The tray is  $3\frac{3}{4}$  inches by 8 inches, and is supported  $\frac{1}{2}$  inch above bottom of oven, insuring uniform heating throughout. The loops at ends of tray are  $1\frac{1}{4}$  inch high and arranged so they cannot drop against surface of tray or ends of oven. The stand is 4 inches by 8 inches and for alcohol lamp is  $4\frac{1}{2}$  inches high—for a Bunsen burner it would need to be somewhat higher. An ordinary support with rings answers equally well.

With an alcohol lamp the temperature can be run up to  $160^{\circ}\text{C}$ . in ten minutes and for a given temperature the results are absolutely uniform. I usually run temperature rapidly up to  $160^{\circ}\text{C}$ . and immediately remove cover and flame and allow oven to cool. The whole procedure requires less than fifteen minutes.  
Burlington, Wis. W. A. FULTON.

## A Convenient Microscope Table.

There has been a demand for a low-priced, movable microscope table for use in the home of the amateur microscopist, and in the private laboratory of the professional worker. To meet this demand, some of the microscope manufacturers include in their catalogues ready-made tables for the purpose. The revolving, adjustable tables as a rule are for temporary use only, and have no place for storing accessories, supplies, reagents, etc., while the larger and more elaborate tables of the desk pattern are so expensive as to be beyond the reach of many an humble worker. Moreover, a serious objection to many of these is that the reagent case extends entirely across the back, thus shutting off much of the light, if perchance, it be necessary to place the table against a window. While this extra light may not be essential in viewing the objects through the microscope, it is usually needed in making the preparations, and we cannot afford to lose it.

In the fall of 1900, the writer designed and had constructed a table for a small laboratory. After a year and a half of nearly constant use the table is found to be quite satisfactory, and some of its features are so convenient that a description and figure are herewith given for the benefit of the readers of the JOURNAL. The general plan was copied from a table designed a few years ago by Dr. W. C. Sturgis, formerly botanist of the Connecticut Agricultural Experiment Station, but most of the details are original. The dimensions are as follows:

Length,	48 inches	(120 centimeters).
Breadth,	30 "	( 75 " ).
Height,	28 "	( 70 " ).

The top is glued together and projects over the sides about one and one-half inch. At the right there are five eighteen-inch drawers for apparatus and supplies, the lower drawer having a face six inches and the others three inches in depth. The shelf over the drawers is found very convenient in note-taking. Two shallow drawers, with a face only one and one-half inch deep and located just under the top-board, are very convenient for freshly prepared slides, drawings, etc. The drawer at the left is nine inches long, and I have used it for preparations in glycerine and water, allowing the slides to remain there until the water has evaporated, when they can be cleaned up and sealed. The middle drawer is fifteen inches long, and is used for sheets of notes, drawings, or blank

#### A Convenient Microscopical Table.

paper. All drawers and the shelf extend to the back side of the table, which forms a stop for them. On brackets at the left end of the table, is a shelf six inches wide and about four and one-half inches below the top of the table, for use in making camera-lucida drawings. The exact height of this shelf depends upon the microscope, as the stage should be perfectly level and even with the top of the table upon which the drawings may be made.

Under the shelf towards the back is a small compartment, provided with lock and key, for storing the microscope and case when not in use.

The table was constructed of North Carolina pine sheathing by a local builder at a cost of about twenty-five dollars, but if made in the factory in considerable numbers, it could doubtless be made of better wood for a smaller price. The

flat top permits the table being placed against a window, and the reagent bottles may be arranged at the sides so as not to interfere with the light. A large pane of window glass makes a fairly good surface upon which to place the microscope and make the preparations. I use a sheet of manila paper under the glass, with small squares of black and white paper at the front to aid in seeing the different-colored objects when mounting them.

The illustration shows the table with all of the features mentioned.

Agricultural Experiment Station, New Haven, Conn.

W. E. BRITTON.

### Marble Blocks for Celloidin Tissues.

The micro-chemical color reaction of tissues embedded in celloidin and mounted on resinous deck-plugs or cork, suffers constant deterioration in the alcohol preservative; the deleterious action of the soluble resins renders pine wood particularly in-apt for blocking celloidin-mass. Though its use is ill-advised, it is still adhered to on account of availability and cheapness. As no means yet devised can restore to celloidin sections, thus affected by the resins, their pristine color-reaction, the use of wood or cork is strictly limited to temporary mounting; at least this should be so. For permanent preservation on the block nothing serves better than *mosaic* cubes of domestic or Italian marble. The mosaic "squares" may be had in various sizes; three-quarters of an inch square by half an inch thick run about thirty to the pound, and cost five cents; four surfaces of the cubes are plane and unpolished and the celloidin adheres perfectly. After a year's trial the author is satisfied that the marble squares possess every advantage of the expensive vulcanized fiber blocks.

Chicago Homeopathic Medical School.

EDWARD CLARK STREETER.

### A New Colony Counter.

From a design submitted to Bausch & Lomb a new bacteria colony counter has been made by which greater accuracy may be attained. The value of the counter is in the fact that the glass bearing the ruled lines may be brought very close to the colonies.

A glass disk ruled to square centimeters is mounted in the end of a short barrel (Figs. 2 and 3 B) that moves freely by screw thread within a collar, C. A block similar to that used in the Barnes dissecting microscope, but of greater size, has a metal circle (Figs. 2 and 3, Cir.)

FIG. 1.—Block without disk or lens.

on the top over the mirror, of a size to hold the 100 mm. Petri dish; a rim is on the circle to hold the dish in position. Underneath the circle a mirror, M, or a black surface if desirable, is placed at an angle of 45 degrees.

A sliding post bearing a jointed arm is set into the block, A, to hold the lens used in counting. The dish to be counted is set upon the circle, the cover is removed, and the barrel is placed, disk down, inside the dish, the collar holding the barrel resting upon the edge of the

FIG. 2.—Apparatus ready for use.

dish. The barrel is lowered through the collar by means of the screw thread until the ruled glass is close to the gelatine. The barrel is of sufficient length that the ruled glass may be brought close to the gelatine in dishes of various depths. By means of the jointed arm the lens is swung into place and may be carried over the entire surface of the dish.

An error in counting is easily made when the ruled glass rests upon the edge of the Petri dish, because the eye in looking between the lines sees an area greater than 1 sq. cm. Colonies almost underneath a line may very easily be counted in each of two areas. By bringing the ruled glass close to the gelatine the cause of such an error is eliminated. The lines on the disk are on the side toward the colonies so they may approach very near.

The barrel and collar are made of such a size that they may be used in dishes that vary a little in diameter. If a dish with colonies is found to be so small the disk will not go inside, then the latter may be set on top and the count made as with the Wolfhuegel apparatus. Also, if at any time it should be desirable to make a count without removing the cover of the dish, then the barrel may be placed upon the table with the glass disk uppermost, the dish set upon it and the count made as with the Lafar apparatus.

FIG. 3.—Barrel in collar, leaning upon edge of Petri dish.

By sorting the Petri dishes already in the laboratory and by asking dealers to supply dishes that do not vary much from the standard size, no difficulty will be met in the use of the apparatus.

Drake University.

L. S. Ross.

## Incubator for the Maintenance of Constant Low Temperatures.

Some time ago we published a description of an incubator intended to maintain a temperature of  $20^{\circ}\text{C}$ .<sup>1</sup> Certain inconveniences which the use of this

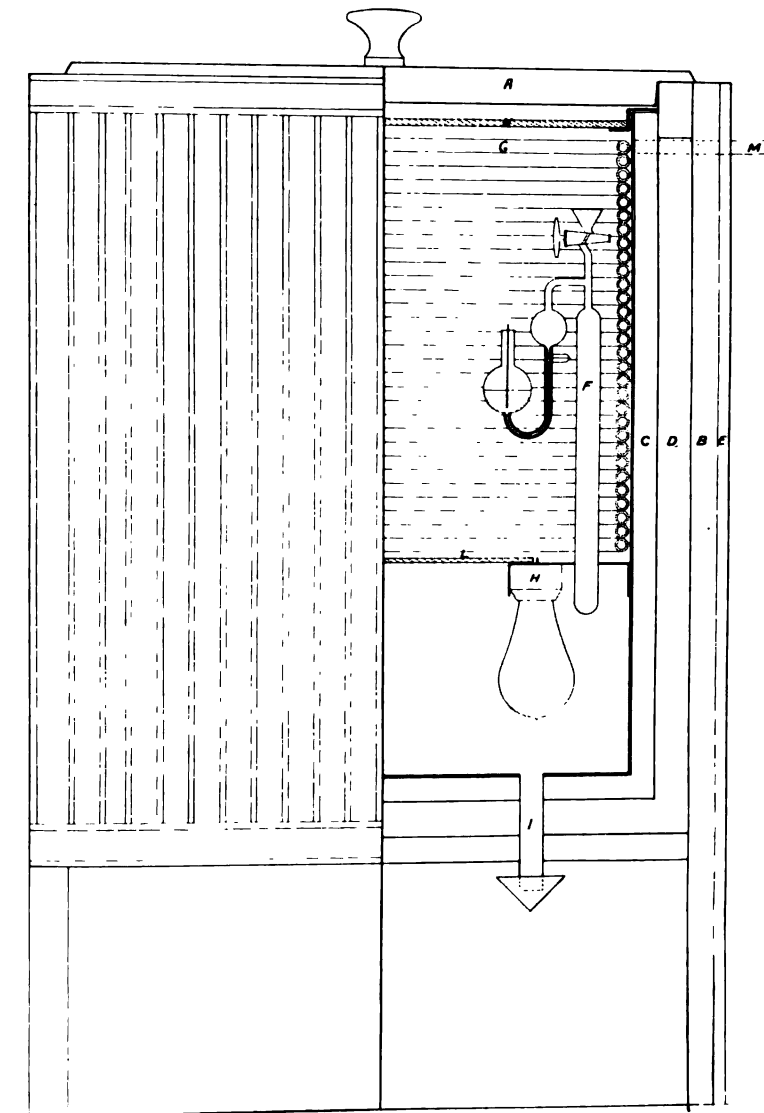


FIG. 1.

apparatus entailed caused us to construct a simpler one. This has been running for a considerable time and has proven satisfactory, the temperature variations seldom exceeding one degree.

<sup>1</sup> Brooklyn Medical Journal, February, 1899.

The incubator (Fig. 1) is a wooden box 22.5 x 20 x 27 inches, made by a local carpenter, and consists of two layers of seven-eighths boards (B and C) with an inch space between them (D), which is lined with building paper and filled with sawdust. Externally the box is covered with a layer of paper over which is a layer of three-eighths matched boards (E). The inside is lined with zinc. The box is supported by extensions of the sides and back, which raises the bottom eight inches from the floor, leaving a space for a drip pan. The door (A), made of two thicknesses of wood with paper between, is at the top. Below this is a removable galvanized iron frame carrying a sheet of plate glass (K). The chamber is divided into two parts by a movable partition (L) of galvanized wire gauze. Around the walls of the upper chamber and entirely covering them is coiled a quarter-inch thin walled lead pipe (G) having inlet and outlet at (M) which carries water for cooling purposes.

Heat is supplied when necessary by a 16 candle lamp, the bulb of which is blackened. This lamp (H) is situated in the lower chamber and at a considerable distance from the regulator. The lamp is turned on or off as required by

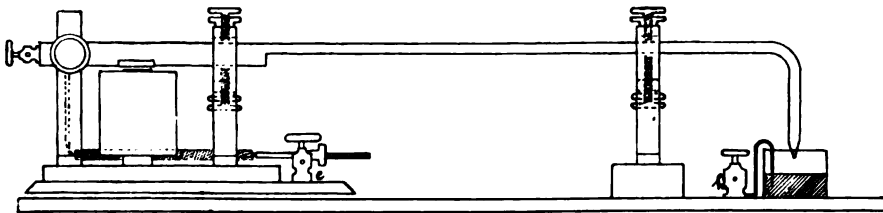


FIG. 2.

a contact maker (Fig. 2) controlled by the regulator (E). The contact maker is a modified telegraph sounder, the arm of which has been lengthened in order to obtain the motion necessary to snap out the arc formed on breaking the circuit. Current for the contact maker is supplied by a battery of four gravity cells in series. The regulator (F) is constructed to close the circuit when the temperature falls below a predetermined point. It contains mercury in the arm and lower small bulb, the other bulbs containing air only. Contact is made through the mercury, one terminal being immersed in the mercury in the lower bulb, the other sealed into the arm. The regulator is adjusted by letting air into or out of the large bulb through the stopcock. This stopcock should be selected with special care, as it must be absolutely gas tight.

The working of the apparatus is as follows :

Water from the city supply is allowed to flow continuously through the lead coil. When the temperature falls below the point at which the regulator is adjusted, contact is made in the regulator closing the circuit through the lamp, which stays lit until the temperature rises to the required point. When water can be obtained directly from the street main it is usually cool enough. If its temperature becomes too high to properly cool the chamber, the water is cut off entirely and a piece of ice placed in the lower compartment.

Hoagland Laboratory.

E. H. WILSON.

R. B. FITZ-RANDOLPH.

### Note on a Method of Cultivating Anaerobic Bacteria.

There is a very excellent method of growing anærobes which I have not seen mentioned, or have overlooked in Hunziker's recent articles in the *JOURNAL OF APPLIED MICROSCOPY*; a combination of the pyrogallol method and a vacuum. Plates or tubes are placed in a bell jar with stop-cock at the top (Fig. 1) and this is connected with a vacuum pump. Pyrogallic acid is placed on the floor

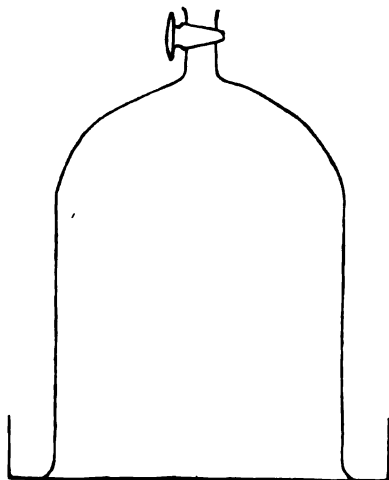


FIG. 1.—Bell jar sealed to dish with paraffin.

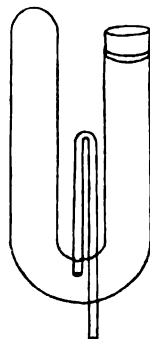


FIG. 2.

of the dish and the illustrated glass apparatus (Fig. 2), which can be easily made by any one familiar with blow-pipe work, inserted, after being filled with KOH or NaOH. The vacuum causes the NaOH in the tube to syphon off and the last remaining trace of oxygen is absorbed.

F. C. HARRISON.

Ontario Agri. College.

### An Easy Method of Demonstrating Gas Production in Test-Tubes.

A fair sized piece of absorbent cotton is placed in each test-tube of sugar (lactose, glucose, etc.) bouillon. The cotton should not be forced to the bottom of the tube or packed tight. The tubes when sterilized are ready for use and are inoculated in the ordinary manner. Gas formed by the growth of the bacteria in the bouillon is caught among the fibres of the absorbent cotton and the gas bubbles, whether large or small, are very clearly demonstrated.

Ontario Agri. College.

F. C. HARRISON.

## Bacteria in the Soil.

Toward the close of the course in Bacteriology at Cornell Medical College, the subject of the soil bacteria is taken up, the students being given some garden earth to plate out, count the colonies, and estimate the number per gram. They are also told something about the Pathogenics found in the soil, but it seemed that, in addition to this, it would be of interest to them to get an idea of the various bacterial processes going on in the soil whereby the cycle of life is maintained, and to this end the following plan was evolved.

In the course of the previous exercises the students are shown how to test for nitrites with sulfanilic acid and naphthylamine (Griess-Ilovsay solution), and for ammonia with Nessler's solution, in order to determine if the bacteria they are studying are denitrifiers or not. The cultures are grown in the following solution :

Peptone,	-	-	-	-	-	-	5 grams.
KNO <sub>3</sub> ,	-	-	-	-	-	-	2 grams.
NaCl,	-	-	-	-	-	-	2.5 grams.
Tap water,	-	-	-	-	-	-	1000 c. c.

After 6 or 7 days in the incubator the cultures are boiled for safety, 3 c. c. measured into an empty test-tube, and to this 2 c. c. of Griess-Ilovsay solution added. A red color shows the reduction of nitrates to nitrites. To the remainder of the culture a drop of Nessler's solution is added; a yellow color showing the presence of ammonia to which the nitrites may have still further been reduced.

Control tubes of the original solutions are, of course, also tested. The students then understand beforehand the tests for nitrites and ammonia.

To demonstrate the process of nitrification in the soil, the following solution is made up 6 or 8 weeks previously :

Microcosmic salt,	-	-	-	-	-	2 grams.
Potassium chloride,	-	-	-	-	-	1 gram.
Sodium chloride,	-	-	-	-	-	1 gram.
Magnesium sulfate,	-	-	-	-	-	.5 grams.
Water distilled,	-	-	-	-	-	1000 c. c.

This is distributed into several Erlenmeyer flasks, about 50 c. c. in each, and sterilized. Into some of the flasks a few grams of garden earth are introduced, and kept in the dark at room temperature. The inoculated flasks are tested at intervals for nitrites, and when these are found to be present in considerable quantities—the maximum is usually reached in 2 or 3 weeks—one-half the flasks are boiled, in order to stop further action, and can then be kept indefinitely, for demonstration of nitrosification.

In the remaining flasks the nitrites gradually become oxidized to nitrates by the nitrifiers, and in 6 or 8 weeks will give no greater reaction for nitrites than the controls, and may be tested for nitrates. For this, evaporate a little of the solution to dryness in a watch glass, add a few drops of phenol-sulfonic acid, and then a little 10 per cent. NaOH solution. A yellow color indicates the presence of nitrates.

We select them for our class: 1. A control flask. 2. One to show oxidation of ammonia to nitrites. 3. One to show oxidation of nitrites to nitrates.

By way of preliminaries to the demonstration of fixation of nitrogen some alfalfa plants are started growing 2 or 3 months beforehand, and the proceedings are as follows:

River sand is thoroughly washed, dried, and sterilized at 150°C. for two hours. Then put into garden pots (we used tin cases in which photographic platinotype paper had been stored), and boiled for an hour or so in the steam sterilizer. The alfalfa seeds are sterilized in 5 per cent. formalin for 15 minutes, washed in two or three changes of sterilized water, sown in the sand, and kept watered with sterile mineral solutions, containing no nitrogen, the one used by preference being:

Tricalcic phosphate,	-	-	-	-	.5 gram.
Calcium sulfate,	-	-	-	-	.5 gram.
Sodium chloride,	-	-	-	-	.5 gram.
Potassium sulfate,	-	-	-	-	.5 gram.
Magnesium sulfate,	-	-	-	-	.5 gram.
Iron sulfate,	-	-	-	-	Trace.
Water,	-	-	-	-	1000 c. c.

One-half of the seeded pots are supplied with pure cultures of nitrogen fixers,

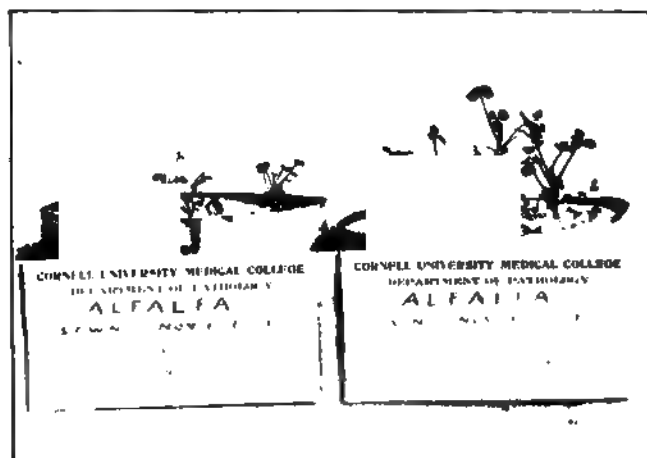


FIG. 1.—Left.—Alfalfa Grown in Sterile Sand. Right.—Same with Cultures of Nitrogen Fixers.

isolated from alfalfa rootlets, two or three times during the first two or three weeks of growth, the remaining pots not being treated.

No precautions need be taken to prevent air bacteria from falling in during the period of growth, but it is advisable to keep the untreated pots in a separate room where no cultures of nitrogen fixers are handled. Conditions, however, of lighting and temperature should be the same for both.

The photographs (Figs. 1 and 2) show the appearance three months after sowing. It is true the growth is not very luxuriant in the treated pot, but a

laboratory window in the winter is not a favorable spot for plant life; and the difference between the two pots is sufficiently well marked for demonstration.

It is easy to obtain pure cultures of the nitrogen fixers. The nodules are cut out from the rootlets, sterilized in the same way as explained for the seeds, pulverized, and the fragments plated out in gelatine. In 4 or 5 days the round non-liquefying colonies appear and can be transplanted. The bacilli will grow on ordinary media, but a specially favorable one is that recommended by Mazé. (Ann. Pasteur, 1897, '98, '99.)

Allow 100 grams dry weight of beans to germinate on moist cotton for two or three days, make an infusion of them in 1000 c.c water at 100°C. for 30 minutes; strain through cloth, add  $\frac{1}{2}$  per cent. NaCl, and neutralize. Boil one hour, filter, and add 3 per cent. of saccharose. The liquid comes out fairly clear and can be used as broth or can be made up with 10 per cent. gelatine.

On bean gelatine slants the bacteria grow in great profusion, and according

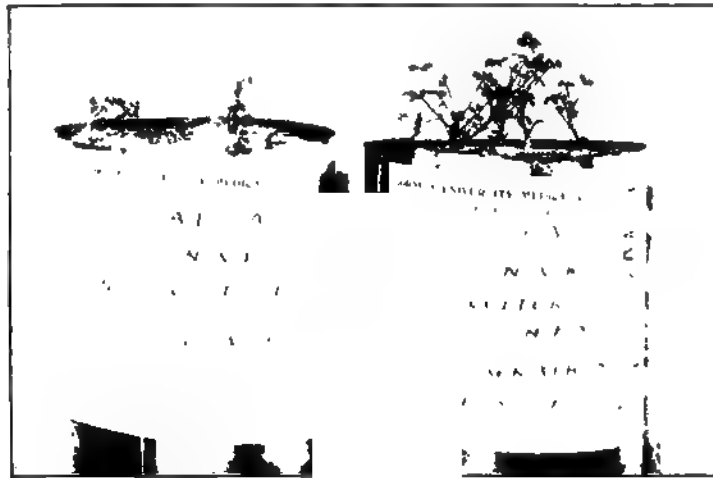


FIG. 2.—Left.—Alfalfa Grown in Sterile Sand. Right.—Same with Cultures of Nitrogen Fixers.

to Mazé they are able to fix nitrogen under these conditions, but this has not been tested by us for confirmation. Most investigators have been unable to get the bacteria to fix nitrogen in artificial cultures.

In the lecture attention is drawn to the fact that there is a constant accession of dead animal and vegetable matter to the soil, and this is disposed of by bacteria; the final products of the carbo-hydrates being gases and water, and of the nitrogenous substances, gases, water, and nitrates, the latter remaining in the soil.

In the course of explanation the following table is built up on the board:

	Changed to	By	By process of
1.—Insoluble proteids	Soluble organic substances	Anærobics	Hydrolysis
2.—Soluble organic	Gases, water and ammonia	Anærobics	Deoxidation
		or	(Putrefaction)
		Facultatives	Deoxidation or oxidation
			(Decomposition)
3.—Ammonia	Nitrites	Nitrosifiers	Oxidation
4.—Nitrites	Nitrates	Nitrifiers	Oxidation

At this point nitrification is demonstrated by means of the flasks prepared beforehand. Small portions of all three flasks are tested in tubes for ammonia and nitrites, and in watch glasses for nitrates; the differences between them

being pointed out, and explanations given as to how the flasks were treated. The photograph (Fig. 3) shows: 1. Control. 2. Nitrosification. 3. The nitrites all oxidized to nitrates.

"Now green plants, speaking generally, can only utilize nitrogen when it is in the form of nitrates, so in this way the cycle of life is completed.

But on the way round there is considerable loss of nitrogen; much passes off as free N during the reduction of soluble matter to ammonia, and the same occurs to a great extent by the action of the denitrifiers. All the nitrogen therefore does not come back to the green plants in the form of nitrates and this loss must be made up somehow. Plants,

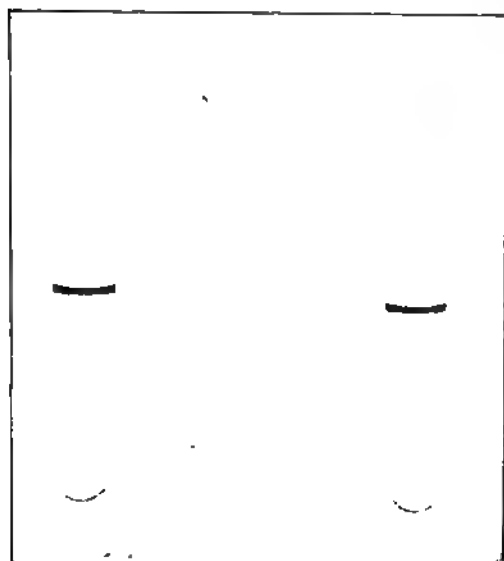


FIG. 3.—Center.—Nitrite Reaction with Griess-Ilosvay Solution. Left and Right.—No Reaction.

animals, and bacteria we have so far considered can use and waste nitrogen, but they cannot fix it from the air. Nor is there in nature any fixation of nitrogen by chemical processes—or at least only to a very limited extent.

There is, however, a class of bacteria which can fix free N from the air under special circumstances, and hand on their nitrogenous surplus products to be utilized by certain green plants,—the leguminosæ, among which are peas, beans, clover, locust trees, etc. These bacteria are called nitrogen fixers and are universally present in the soil.

The seed of a leguminous plant when sown contains enough nitrogen to start the young plant growing. The stem shoots up a little way and the rootlets penetrate the soil to a consid-

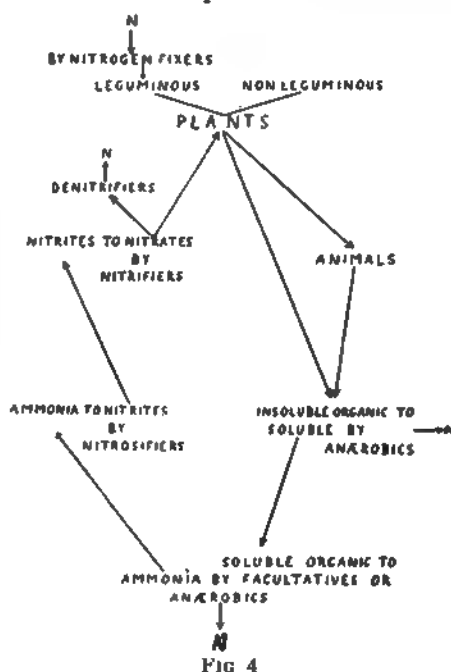


FIG 4

erable depth before the young plant feels the necessity for more nitrogen. As the rootlets extend, the nitrogen fixers penetrate into them, and, growing there, form small nodules, which gradually increase in size as the bacteria multiply. See photograph (Fig. 5) of nodules on roots of locust tree.

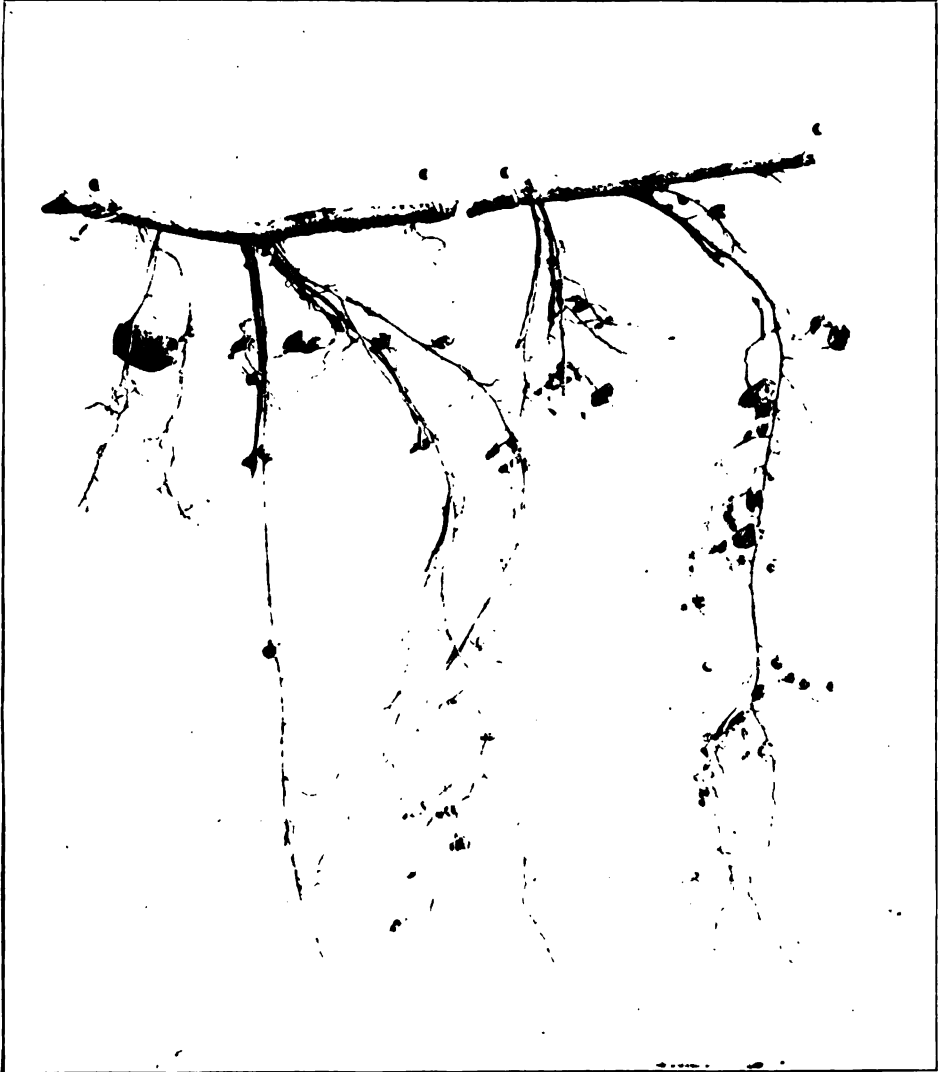


FIG. 5.—Nodules on Roots of Locust Tree.

At first the bacteria grow at the expense of the plant, but when once they have made a good start they become able to make use of the free N of the air, forming certain nitrogenous products. Whether these products are in the form of nitrates or some other compound, is not yet determined, but at any rate the plant can utilize them, obtaining in this way nitrogen necessary for its growth.

The plant continues to supply the bacteria with carbohydrates, which it can build up from  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , and in return receives the surplus nitrogen of the bacteria.

Leguminous plants, when they die, always return more fixed nitrogen to the soil than they take away from it, and in this way the cycle of life is perfected."

The plants are then shown to the class and cultures of nitrogen fixers are provided for examination.

B. H. BUXTON.

Cornell University, Medical College.

## A Method for Fixing and Sectioning Bacterial Colonies, Fungus Mycelium, Etc.

In determining the manner of growth of some bacteria, I have occasionally found it desirable to section an entire colony. This can be accomplished by the following method, which is equally well applicable to the sectioning of fungus mycelium:

Grow the bacteria or fungus in nutrient agar in a Petri dish. One per cent. agar is sufficiently dense, and enough should be poured into the dish to give a thickness of about 2 to 4 mm. When growth has proceeded to the desired stage, cut out blocks of the agar from 3 to 6 mm. square, and drop them into fixing fluid. I have used Flemming's, Hermann's, and the mixture described below, with good results. Wash, dehydrate, and embed as for any tissue, keeping in mind that the agar block is rather difficult to penetrate, and the time for dehydrating and infiltrating must be increased to two or three times its usual length.

Where it is necessary to "set" a bacterial colony to prevent the washing off or diffusion of the bacteria, a fixing fluid composed of 50 c. c. absolute alcohol, 30 c. c. chloroform, and 15 c. c. acetic acid, I have found to be very satisfactory, even where very delicate fixation was desired. Fix tissues four to twelve hours. Tissues must then be washed in alcohol, and it is best to make the transition from fixing fluid to absolute alcohol gradually. That is, pour out about one-third of the fixing fluid and add about an equal quantity of 95 per cent. alcohol; allow the tissues to stand a few hours in this mixture, pour out more and add more alcohol, and finally transfer to absolute alcohol.

KARL KELLERMAN.

Bureau of Plant Industry, Washington, D. C.

## Some Apparatus Used in the Laboratory of the Buffalo Department of Health.

### APPARATUS FOR USE IN SPUTUM INVESTIGATIONS.

A specially designed box (Fig. 1) containing the bottle (Fig. 2) for the collection of sputum is used, and when it is returned to the laboratory it is placed in one of the compartments in the Sputum Box Container. When ready to be

**DEPARTMENT OF HEALTH,  
BUFFALO, N. Y.**

# SPUTUM OUTFIT

*For submitting samples to be examined for Tubercle Bacilli.*

*These Sputum Outfits can be  
Returned to any Police Station or  
Direct to the Department of Health.*

*A report will be mailed the  
Physician as soon as possible.*

### DIRECTIONS.

Remove bottle from pasteboard box before collecting sample of Sputum. Fill bottle one-half ( $\frac{1}{2}$ ) full of Sputum and tightly insert cork. Do not allow any Sputum to get on the outside of bottle. If such should occur, wash off with 5 per cent. solution of Carbolic Acid. Place bottle back into box, put on cover and completely fill out spaces requesting name, address, etc.

Patient's Name .....

Patient's Address .....

No. of City Ward .....

Physician's Name .....

Physician's Address .....

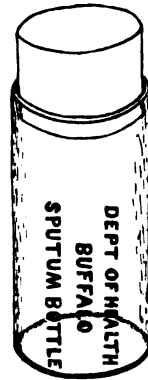


FIG. 2 — Sputum Bottle.

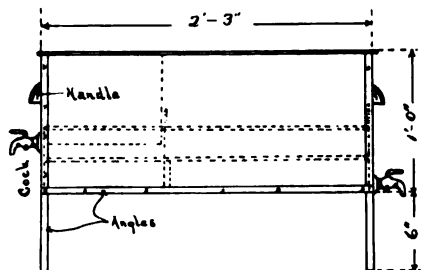


FIG. 1.—Wrapper of Box used in collecting Sputum.  
The box measures  $1\frac{1}{4} \times 1\frac{3}{8} \times 3$  inches.

3-a.—Side View of Sputum Box Container.

examined the entire box is dipped in the bath of carbolic acid (Fig. 3). In this way the box and its bottle are disinfected before being opened. Investigation has demonstrated that at least 25 per cent. of all bottles sent in having been filled with sputum by patients contain sputum on the outside of the bottle. By the method described the danger of infection to those working in the laboratory is minimized.

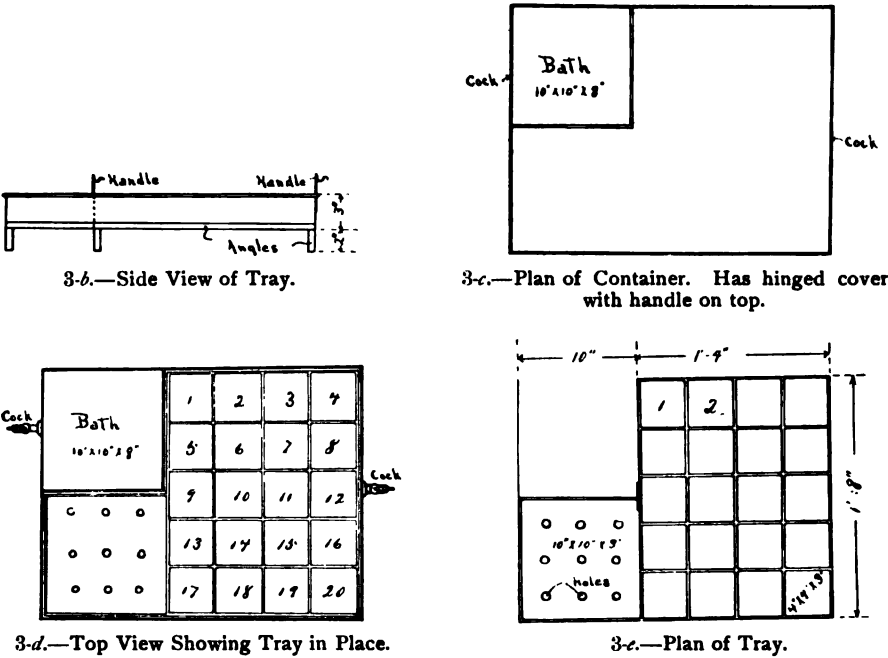
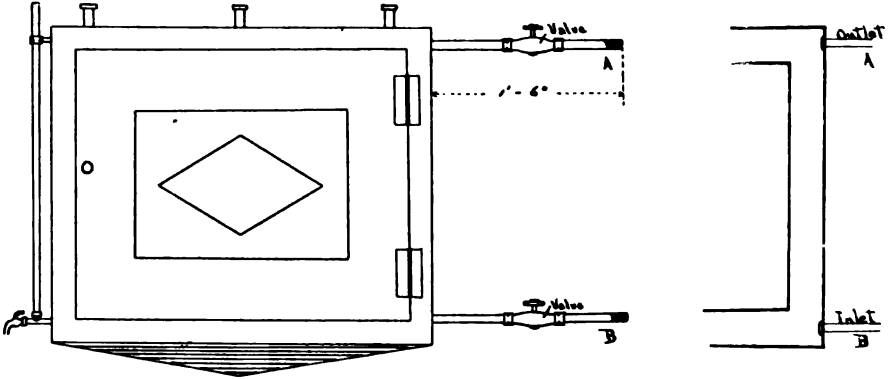


FIG. 3.—Sputum Box Container. Made of copper, reinforced with angle iron, and air tight. Tray is made of stiff zinc, reinforced with angle iron.

INCUBATOR FOR USE IN WATER ANALYSIS.

It is necessary during the various months of the year to have some sort of an appliance in the nature of an incubator in which plates of water samples in gelatin will grow at a temperature from 20 to 24 degrees C. The diagram pre-



Incubator for Use in Water Analysis. A and B are openings entering the water jacket, into each of which is brazed a  $\frac{1}{2}$ -inch brass pipe  $1\frac{1}{2}$  foot in length. In the center of each pipe is a brass valve.

sented is a Bausch & Lomb incubator modified so as to permit of a constantly running stream of water through the water jacket from bottom to top. The temperature of the instrument is regulated in the ordinary manner by a low

temperature thermostat controlling a burner underneath. During seasons of the year when the tap water is below the temperature required the water is run directly into the incubator, but during the summer months, when the temperature of the tap water itself is above 20° C., the water is first run through a bath of shaved ice by using a coil in the ice, and then to the incubator.

The instrument is subject to a variation of never more than three degrees, which, in the designer's experience, does not materially influence the results in water plate work with gelatin. At times there is considerable condensation of moisture on the interior of the incubator. This can be easily overcome by using a layer of coarse asbestos on which to place the plates. Coarse blotting paper acts equally as well. After many attempts with other devices the one described answers better than any other tried.

The water incubator is best placed over or near a sink so that the constantly running water, which at times is but a very small stream, has a free outlet. The amount of water to be supplied to the incubators varies with the season of year, and depends entirely on the water temperature as it enters the water jacket.

Dept. of Health, Buffalo, N. Y.

WILLIAM G. BISSELL.

### A Fixation Method for Hydra.

The common fresh water Hydras (*Hydra fusca* and *H. viridis*) do not present unusual difficulties to the preparateur, and yet perfect results are not always obtained. The tentacles, especially, are difficult to manage. If fixed in a well-extended condition, as they should be, they not infrequently become twisted together, and are then extremely liable to be broken off in subsequent transfers with pipette or spatula.

The following method does away with this difficulty. It was employed by the writer some years ago in the preparation of *Hydra fusca* in large numbers for class-study, and found entirely satisfactory. Recently I have applied it to *Hydra viridis* with equally good results.

The Hydras are transferred from the aquarium with a pipette and placed singly in watch-glasses containing just enough water to cover them. They soon attach themselves and expand. Either picro-acetic or aceto-sublimate may be used as a killing agent. No doubt other standard fixatives will serve as well. Whatever fluid is used it must be heated nearly to boiling and applied precisely as indicated.

When the Hydra is well expanded in the shallow layer of water, its position is necessarily almost horizontal. A pipette-full of the hot fixing fluid is suddenly squirted into the watch-glass so that the current sweeps along the Hydra from its aboral to its oral extremity. It is thus instantaneously immersed in the fixing fluid, in which it stiffens out and dies in an extended condition. The tentacles are stretched out in a straight line coinciding with the principal axis, while their tips are slightly approximated. In this position there is little danger of their breaking off in reasonably careful manipulation.

The moment the Hydra is killed the watch-glass is filled with the hot fixing

fluid, in which the specimen is left for an hour or so if picro-acetic is used, but only 5 to 10 minutes in aceto-sublimate. The washing-out in case picro-acetic or aceto-sublimate is used is invariably done with 50 or 70 per cent. alcohol to which after aceto-sublimate a few drops of alcoholic solution of iodine are added to complete the extraction of sublimate.

The best stains for entire mounts are borax carmine, alum carmine, and alum cochineal. Hæmatoxylin is liable to give an overstain which is difficult to extract. Both hæmalum and Czokor's alum cochineal are excellent for staining Hydras it is intended to section.

The aceto-sublimate consists of a saturated aqueous solution of corrosive sublimate, to which five per cent. by volume of glacial acetic is added. The picro-acetic contains one part of glacial acetic to ten or more parts of saturated aqueous solution of picric acid. Exact proportions do not seem to be important, but it is well to remember the swelling action of acetic acid on all tissues and avoid an excess. Slight swelling of specimens to be mounted *whole* is rather an advantage, inasmuch as it renders them more translucent and the cellular structure more easily seen. It should be avoided for specimens that are to be *sectioned*. For the latter I have found aceto-sublimate a better fixative than picro-acetic.

HERBERT P. JOHNSON.

West Roxbury, Mass.

## Vegetable and Animal Cells.

Most workers in biological science, especially young teachers, realize how difficult it is to provide simple yet comprehensive material from which the student may get his first conception of the structure of the vegetable and animal cells. The lesson is one of great importance, as all will agree, since it involves the morphological unit of animal and vegetable structure.

In this lesson, following closely the one on the use of the microscope, besides learning the integral parts of the cell, the student should learn something of self-reliance in manipulation, especially that which pertains to simple fixing, selective staining, washing and clearing. For this reason, mounted slides should be kept in the cabinet and only brought out to supplement what the student will so interestingly have discovered for himself.

The onion, or onion bulb, is always easily secured, and from it may be obtained the material for the study of the vegetable cell. The onion should be cut into halves and a piece given to each student. He is then directed to separate the layers with a pair of forceps. Clinging to each scale is a delicate vegetable membrane. This is carefully lifted off with the forceps and with shears is snipped into pieces as large as the end of a lead pencil. These pieces are immersed for three minutes in 30 per cent. alcohol. This is to *fix* them.

While the above pieces are fixing, a single fresh piece may be mounted in water. This will show in faint outline the nuclei and cell walls, giving the student an idea how the specimens appear unstained.

The student is now ready for staining. Two of the above fixed specimens

are placed for five minutes in a 1 per cent. watery solution of iodine, rinsed and mounted in water. This procedure stains the nucleoli, nuclei, protoplasm, and cell walls a beautiful yellow or brown.

The beginner is likely to be in doubt over the protoplasm of the cell. An experiment in plasmolysis will give him a better idea of its nature. Two or three of the fixed pieces are treated for five minutes with an 80 per cent. solution of alcohol and then stained for three minutes in a 1 per cent. watery solution of methyl violet, rinsed and mounted in water. This gives a beautiful violet preparation showing not only the cell wall, nucleus, nucleolus, but also the protoplasm as a distinct mass with a clear space between it and the cell wall.

A beautiful double stain may be secured as follows: Two or three pieces of the fixed membrane are immersed in Delafield's hæmatoxylin for ten minutes. They are then rinsed in water to free them from an excess of stain. The hæmatoxylin is then removed from all parts of the cell except the nucleus by the following mixture: Hydrochloric acid, ten drops; water, 100 c. c. The stained specimens should lie in this till they fade to a salmon pink in color. They are then to be floated on ordinary water till they assume a light blue color. Now they are ready for a 1 per cent. watery solution of eosin for one-half minute, rinsed and mounted in water. The preparation should show the nucleus stained a rich blue, and the other structures an attractive pink.

Satisfactory work on the animal cell may be secured by the use of smears of frog's or toad's blood on glass slides. The toad is suggested as it is more easily secured than the frog and is every whit as good. The animal is anæsthetized with chloroform. Its chest cavity is exposed and the apex of the heart is snipped off. The blood spreads into the pleural cavity. A swab may be made by twisting about the end of a toothpick a bit of surgical cotton half the size of a pea. Each member of the class makes his own *smears* by a light stroke with the above swab. Blood side up, the slides are passed through the Bunsen or alcohol flame three times. The smear is then ready for the stain.

A drop or two of Delafield's hæmatoxylin is placed on the blood spot and allowed to act ten minutes. The slide is then held under the flow of a tap till all free stain is washed off. There is no danger of washing off the smear. A drop of a 1 per cent. watery solution of eosin is flooded over the smear for one-half minute and then as before it is held in the flow of the tap water till no stain washes away. Glycerine is added and a cover glass.

In a successful mount, the oval blood cells show a most beautiful contrast stain. The nucleus is a deep blue and the protoplasm, without the nucleus, a bright pink. The fact that hæmatoxylin is a nucleoplasmic stain and eosin a cytoplasmic one may be emphasized from the very start.

Slips bearing directions for each step of the above operations should be in the hands of each student at the beginning of the laboratory period.

## A Platinum Strainer for Use with Sections Which Are to be Prepared in Accordance with the Pal-Weigert Method.

It is an extremely difficult matter to prepare very small sections according to the above mentioned method. The difficulty arises from the fact that eleven

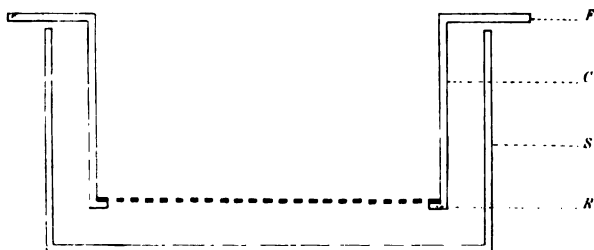


FIG. 1.

changes of reagents must be made from the time the sections are cut until they are ready to mount. The sections require, at two stages in their preparation, to be washed in several changes of tap water. If one tries to make the required changes

of reagents by careening the dish in which the sections are immersed and allowing the liquid to pass out over them, many of the small sections go over. If one tries to make the various changes by transferring the sections individually, the task becomes almost endless.

The following piece of apparatus, shown in cross-section in Fig. 1, has been used with success in the Neurological laboratory of the University of Chicago.

The apparatus consists of an ordinary stender dish (S, Fig. 1), 60 mm. in diameter and 30 mm. in depth. A glass cylinder (C), 50 mm. in diameter and 25 mm. in depth, fits into the stender dish. This cylinder has a wide flange (F) extending around its top edge. The flange makes it easy to transfer the cylinder from one stender dish to another. The bottom of the cylinder is drawn in horizontally for 1.5 mm. forming a rest (R) for the platinum strainer (P). This strainer (shown in bottom view in Fig. 2) consists of a piece of medium weight, perforated platinum foil (platinum was used because it resists the action of the stain and the differentiating fluids). The perforations are made  $\frac{3}{4}$  mm. in diameter and 2.5 mm. apart.

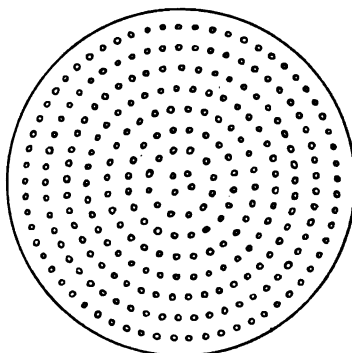


FIG. 2.

The method of using the strainer is as

follows: The various reagents are put into stender dishes arranged side by side. The cylinder containing the sections is then transferred from one dish to another. It is not necessary to touch the sections from the time of sectioning until they are cleared and ready to mount.

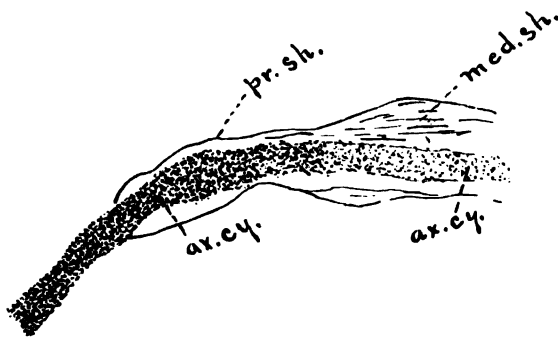
(Cf. Schaffer, *Zeitschrift für wiss. Mikroskopie*, 1899, p. 422.)

University of Chicago.

JOHN B. WATSON.

## Staining Axis-Cylinders of Fresh Spinal Chord.

The convenience of the method here described for the demonstration of the structure of the medullated nerve fiber is the justification for presenting it. I do not recall having seen just this method recommended, but it has doubtless occurred to many; it may, however, interest some. The material used was a part of the spinal chord of a calf obtained at the butcher's. Though the calf had not been just previously killed, still the structure of the nerve had not deteriorated seriously enough to interfere with the demonstration. I have found that butcher's meat, very likely as a result of its refrigeration, is often quite adequate for use in making out many of the coarser points in histology and much more convenient than any other source of material. A small piece of the white part of the chord is placed in 30 per cent. alcohol and kept in an oven at 56°C. for 6 hours. After this maceration, which loosens the fibers from one another, small pieces are removed to a glass slide and teased apart in distilled water, and a cover-glass



Stained Axis-cylinder of Fresh Spinal Chord.

is placed on them. The fibers are now separated and at places ends are seen running out into the water. An aqueous solution of acid violet (Gruebler's) is now made by adding to a few crystals of the dry stain a few drops of distilled water. The strength of the solution is not of importance. The mount is now irrigated with this solution and the fibers are

kept under observation during the process. It acts very quickly, and will stain the protoplasmic part of the fiber, i. e., axis-cylinder, almost at once. The stain passes down the axis-cylinder, becoming fainter and fainter. After a short time the action is suspended by irrigating with water, and the appearance shown in the figure results.

In such a preparation there was no difficulty in differentiating the primitive and medullary sheath from the axis-cylinder; the latter is very distinct. The non-protoplasmic nature of the envelopes of the fiber is well shown. It is clear that the sheath has at least stood between the axis-cylinder and the stain, or it may have shut out the stain entirely, the latter having perhaps passed in through the end. The stain fades out very gradually in the deeper parts of the fiber.

I have not attempted to make permanent preparations by this method, it is so easy to make slides that it seems better to work with fresh material. I do not know how long the effect of the stain would last if preparations were kept in glycerine, but I think that it would not be a very permanent preparation.

### Artificial Sap Pressure.

In order to test the effect of sap (water) pressure upon the opening of buds, the following experiment was devised.

A stem or twig of the tree to be tested is inserted into a piece of rubber tubing, which with its connections are filled with water. This forms the closed short end of a U tube. The bend of the U contains just enough mercury to cause the water to overflow before the twig is inserted in the rubber tube. After inserting the stem, additional mercury is poured into the long arm of the U until the desired pressure is produced. The pressure of the mercury forces the water into the tissues of the stem with a force equal to the weight of the mercury above the 0 point on the long arm of the U; the 0 point being the surface of the mercury when balancing the water in the short arm.

Provision is made for restoring the pressure and replacing the water forced into the stem, or lost through it, by inserting a T connection between the end of the U and the base of the stem. The branch of the T is connected with a stout rubber bulb of 75 c. c. to 100 c. c. capacity, and between it and T a stop-cock and an automatic valve are placed.

As now being used, the pressure is maintained at 500 grams as constantly as possible. Poor heat regulation in the laboratory rooms renders considerable variation unavoidable. Twice a day the pressure is

Apparatus for Testing Sap Pressure.

restored, after it has fallen by the movement of water into and through the stem. To restore the pressure, water is forced from the bulb past the automatic valve and the stop-cock into the water space in the short arm, until the level of the mercury is restored to the 500 gm. level. This gives a height of mercury, with the tubing used, of 22 inches above the 0 mark.

The automatic valve (made by fastening a band of rubber across the end of a piece of glass tubing) prevents the return of any water and holds the pressure while turning the stop-cock. By this apparatus, liquid has been forced from the scales of cherry buds at the tips of stems 28 inches long, forming globules of apparently resinous matter upon the surface of the buds. As yet no other effect has been observed. A check stem is kept in a glass of distilled water (used in the experiment) by the side of the apparatus. Moisture was observed on all buds up to 6 inches from tips in 24 hours from setting up experiment on January 27th.

A duplicate of the above was set up on February 2d, using maple in place of cherry. The two U tubes are supported on the same stand, and are filled by the same water bulb. But below the automatic valve, each has its own stop-cock, so that either one may be set independently of the other, or the two set at once and at a common pressure.

DESCRIPTION OF FIGURE.—A, Supply of distilled water for twigs. B, Water bulb for restoring pressure and water. C, Automatic valve, shown separately at C'. D, T-tube supplying water to the two stems. E, E', T-tubes and stop-cocks for each stem. F, Pinch-cock to prevent the return of water when B is compressed. G, G', Stems in place (diagramatic). M, Mercury column. W, Water space.

Maryland Agricultural College.

FREDERICK H. BLONGETT.

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NUMBER 10.

**A Macroscopical Mount for Museum and Class  
Work.**

PREPARED  
CLINICAL  
LABORATORY

Every modern instructor in zoölogy knows the advantage of having numerous prepared specimens for demonstrations. Glass bottles and jars serve well enough for some wet specimens, but in many cases the details of feature and structure are largely obscured by the reflection and refraction of light caused by the shape of the receptacle containing the specimen. Even the rectangular jars so often used are objectionable on account of their cost, and also because they do not easily permit the arranging of a series of specimens in order.

Therefore the method of using a square piece of glass as the base and a watch crystal as the cover has been devised. The material to be mounted should be dissected or fixed in shape for demonstration, and then fastened to the square of glass with a few drops of a solution made

by dissolving ten grams of gelatin in 60 c. c. of hot water. The gelatin will harden sufficiently in about fifteen minutes so that the specimens thus arranged may be immersed in five per cent. formalin, and all sediment carefully washed away.

FIG. 1.—Photograph of the parasitic crustacean of the cod *Lernæa branchialis*, as seen in the crystal mount.

The most satisfactory mounting fluid for general purposes is five per cent. formalin, but for many vertebrate eggs a fifteen per cent. solution is necessary. Only boiled or distilled water should be used in making up the mounting formalin, otherwise air bubbles are liable to be present after the mount is sealed.

The mounting fluid thus prepared should be placed in a jar or pan, and the specimens fixed to the glass, laid therein, so that the cover may be placed and the air completely excluded. The mount is then ready to be raised horizontally from the pan, and the superfluous fluid around the edge of the crystal absorbed with filter paper. A thick solution of balsam dissolved in toluene is then dropped around the edge of the cover, cementing it to the base. The balsam will harden in from two to six weeks, according to the size of the specimen, so that the mount may be handled in any manner.



FIG. 2.—a, Square base glass; b, balsam cement; c, watch crystal cover.

I have used in these mounts crystals varying in diameter from one to ten inches, and of a depth from one-eighth inch to two inches. If the crystals are not ground so as to fit accurately to the base, the edges must be dipped in a ten

per cent. solution of gelatin before being applied.

I have made durable mounts in the last 18 month of sections of the brain, series of the vertebrate brains, dissected eyes and ears, series of fish and frog eggs showing all stages of segmentation, series of vertebrate embryos, and dissections of various systems of the Achordates.

Labels and letters may be attached with warm gelatin solution to any portion of the specimens before immersing them in the mounting fluid. Waterproof ink must of course be used in writing.

A dry mount similar to the one above described serves admirably for displaying insects. In this case a warm ten per cent. solution of gelatin should be used to cement the crystal to the base.

ALVIN DAVISON.

Lafayette College.

## The Microscopical Exhibit at the New York Botanical Garden.

A popular feature of the display intended for public instruction at the museum of the New York Botanical Garden is the permanent microscopical exhibit, made possible by the use of twenty-four microscopes of special design provided through the generosity of Mr. William E. Dodge.

The Leitz stand V is used in the construction of these instruments. The foot is removed and the base of the supporting column is fastened into a hardwood block 6 x 6 x 1½ in. This wooden base is blackened and has beveled edges. The whole instrument, with the exception of the upper part of the draw-tube, is then enclosed in a plate-glass case as indicated in the accompanying figure, the outside measurements of the case being 8½ x 4¾ x 4¾ in.

The glass plates forming the sides of the case are inserted in grooves on the wooden base and are held together at the top by a metal collar. Cement was at

first used at these lateral joints, but is now dispensed with, as it was found that it did not allow sufficient play for the expansion and contraction of the glass incident to changes of temperature in the room. The top of the case is held in position

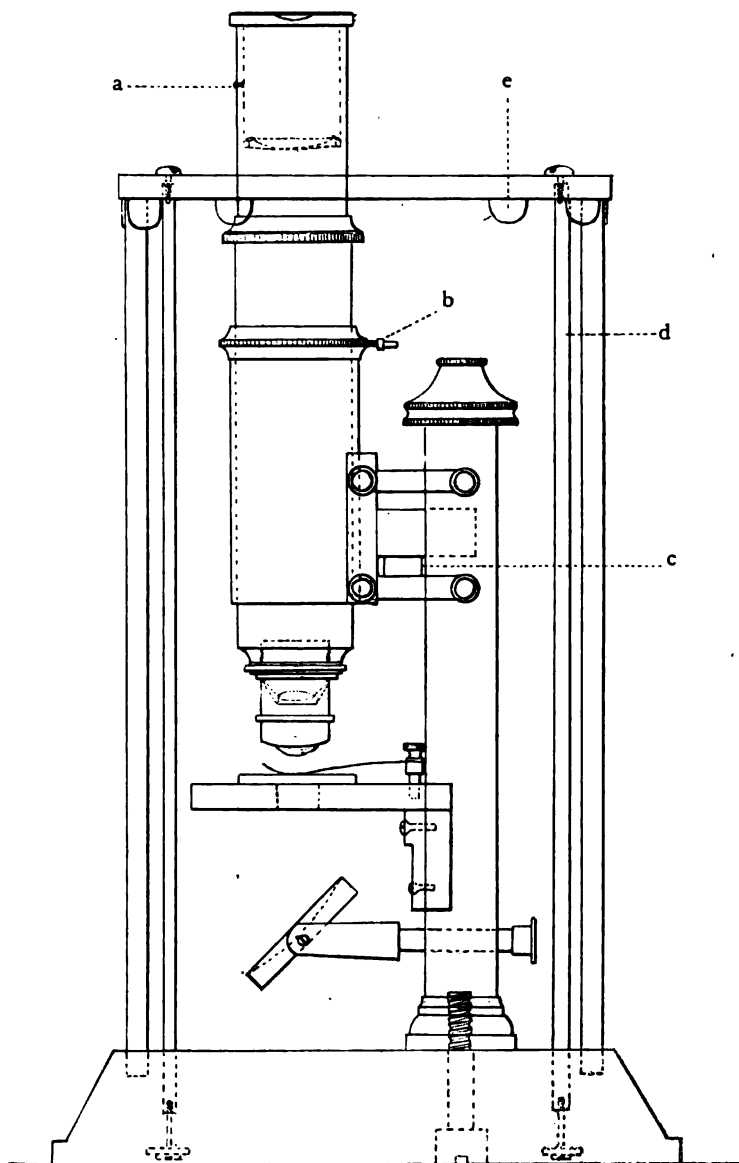


FIG. 1.—An outline of exhibition microscope and lass case; *a*, set-screw for fastening the ocular; *b*, clamping ring for fastening draw-tube; *c*, block of brass inserted between arm and the lower support; *d*, upright metal rod; *e*, flange of metal collar.

by four hollow, upright metal rods which pass through the wooden base and at the top through processes of the metal collar. The top is fastened to the rods by key-screws, with rubber washers, and screws also fasten the rods on the under

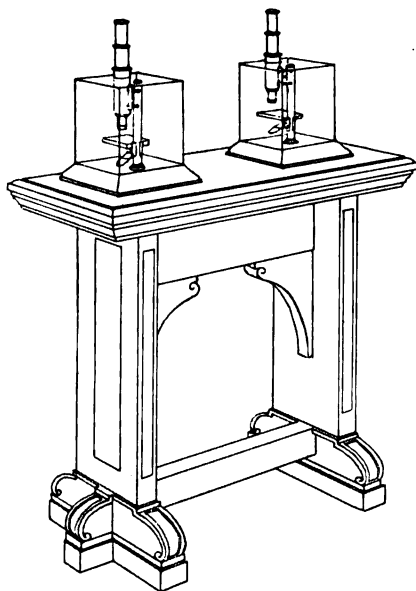


FIG. 2.—Exhibition Microscopes mounted on table. Heavy iron sills in the bases of the tables secure the necessary stability.

side of the wooden base. The aperture in the glass top through which the draw-tube emerges is lined with felt in such a way as to be dust-proof.

The microscope is kept in fixed focus with the aid of a clamping ring at the upper end of the body-tube. The ocular also is fastened by a set-screw. In order to avoid the possibility of forcing the whole microscope-body downward by heavy pressure from above, to the detriment of the exhibition object, an accurately fitting block of brass is now slipped in between the transverse arm and the movable support below. With these precautions, the lenses remain in focus and the chance of accident to the exhibition object is practically removed. Most of the instruments are fitted with ocular II and objective 3, giving a magnification of about 70 diameters.

The objects selected for display at the present time are chosen from among

the lower plants and help to illustrate the exhibits in the neighboring show-cases of the systematic museum. Each microscope is accompanied by a label bearing a moderately detailed explanation of the object exhibited. The preparations on exhibition at the date of writing are as follows: 1. Plasmodium of a slime-mould (*Physarum cinereum*)—stained; 2. Spore-bearing stage of a slime-mould (*Dictydium umbilicatum*)—dry mount; 3. A diatom (*Isthmia nervosa*); 4. A green fresh-water alga (*Draparnaldia*); 5. A green seaweed (*Anadyomene stellata*); 6. A brown seaweed (*Ectocarpus siliculosus*); 7. A red fresh-water alga (*Batrachospermum*); 8. A red seaweed (*Ptilota elegans*); 9. A red seaweed (*Polysiphonia*); 10. A red seaweed (*Ceramium*); 11. Spores of the rose-rust (*Phragmidium subcorticium*); 12. A saprophytic fungus (*Sporormia herculea*); 13. A saprophytic fungus (*Ascobolus*); 14. A saprophytic fungus (*Sarcobolus*); 15. Section of a lichen (*Solorina crocea*); 16. A scale-moss or leafy liverwort (*Frullania Nisqualensis*); 17. Spore-case of a leafy liverwort (*Frullania Bolanderi*); 18. Cross-section of a moss-leaf *Polytrichum commune*; 19. Spore-case and peristome of a moss (*Funaria hygrometrica*)—dry mount; 20. Lid and ring of a moss-capsule (*Funaria hygrometrica*); 21. Peristome of a moss (*Mnium cuspidatum*); 22. Cross-section of the leaf-stalk of a fern (*Polypodium vulgare*); 24. Spores of a horse-tail (*Equisetum sylvaticum*).

MARSHALL A. HOWE.

New York Botanical Garden.

### A Rack for Exhibiting Charts.

While many lecture rooms are fitted with racks for exhibiting charts, it very frequently happens that considerable difficulty is experienced in satisfactorily arranging the charts, especially if they are of different sizes and kinds. The

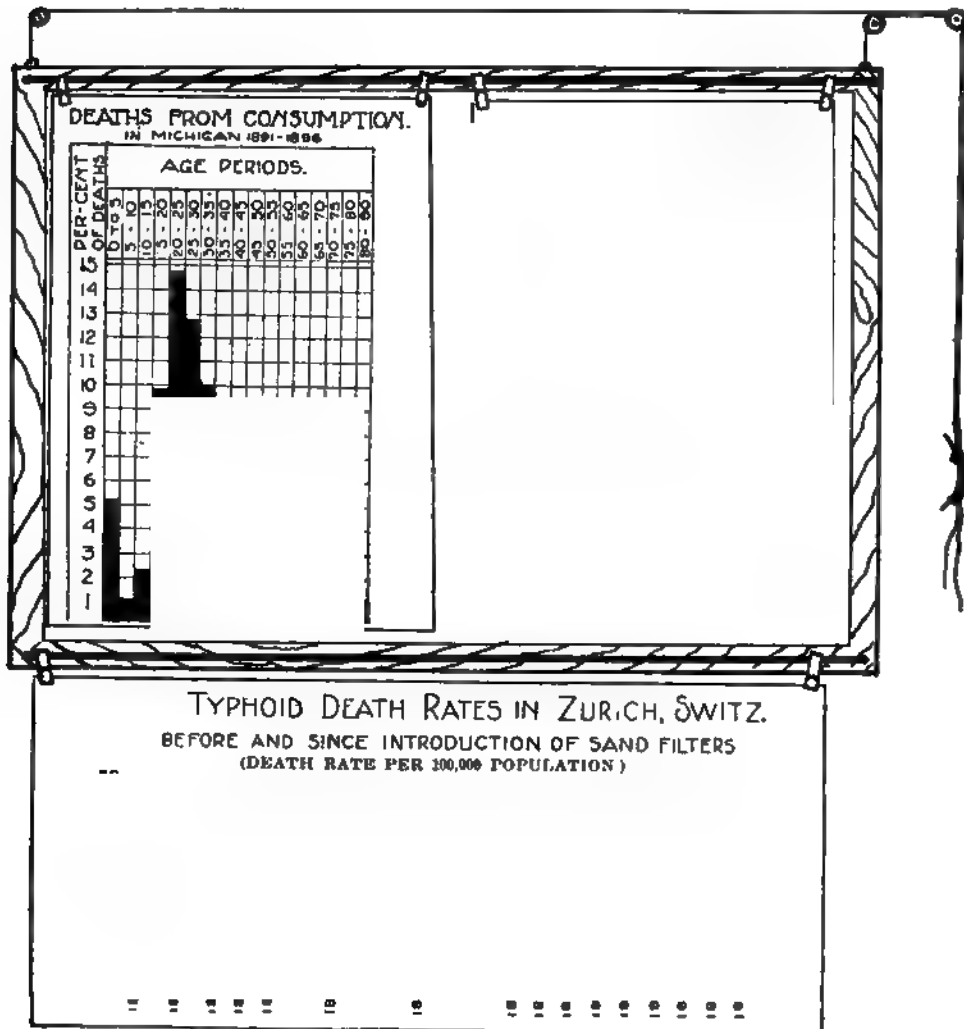


FIG. 1.

rack about to be described has been in use for a year and has been found thoroughly reliable.

The rack is made of any convenient size and consists of a wooden frame and horizontal brass rods, such as are used for hanging lace curtains. These rods may run through the wooden uprights, or preferably, be fastened in front

of the wood by means of the brackets usually sold for the purpose. On these rods are placed a number of "lion-jawed" hooks, which can be purchased at any bookstore, attached to a short length of brass tubing a little larger than the rods. This tubing is soldered securely to the hook. (See Fig. 2.) Mounted in this way the hooks can be easily slipped along the rod to the proper place, but when released by the hand become clamped, due to the weight of the chart, and thus keep the charts in place and "taut." (Fig. 1.) With this arrangement it is possible to keep long muslin charts tight and smooth, something difficult to accomplish except

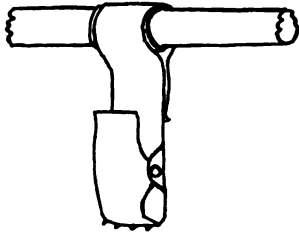


FIG. 2.

where charts are mounted on wooden rollers.

This rack possesses the following good points:

1. It can be made of any size or shape.
2. Materials of construction are all commercial articles.
3. Any sized charts can be used and quickly hung. Charts of different kinds of material and binding can be used at the same time.
4. Muslin charts can be kept "taut."
5. All charts are easily hung and taken down.

University of Wisconsin.

W. D. FROST.

## An Attachment to the Minot Microtome for Cutting Sections of One Micron Thickness.

In 1896 the author designed and constructed a microtome after the Minot pattern with an attachment for cutting sections from 1 to 25 micra thick. It has been used for six years, and has proved so satisfactory that I venture to describe it somewhat in detail. Accuracy, simplicity, and compactness are very essential attributes in any piece of apparatus.

The attachment occupies no more space, and is but little more complicated than the old style Minot. It consists of two ratchet wheels (Fig. 1, A and B) with cog gear wheels attached and so arranged that they may be readily thrown into or out of play. The notches on ratchet wheel A are so cut that each is equal to .005 mm., or  $5\mu$ . Those on ratchet wheel B are equal to  $1\mu$ . The gear wheels C and A are so cut that five notches of B are equal to one of ratchet wheel A. The notches of B are shorter than those of A. It is therefore easy to calculate the distance out on the lever K that the dogs F and E should be placed so that each would move over the same number of notches on their respective ratchet wheels. The wheel J is arranged so that the different positions give from one to five clicks on either wheel. The spring I extending between E and F is sufficient for both dogs.

If one desires to cut sections from 1 to 4 micra thick, E is held out of play by the prop G. Then the dog F will move ratchet wheel B the number of

notches indicated by wheel J. If, however, one desires to cut sections 5 micra thick, then only the dog E and ratchet wheel A are needed. Dog F is thrown out of play by the prop E, and the gear wheel C is moved back out of play with gear wheel A. This is done by an eccentric device shown in Fig. 2. The

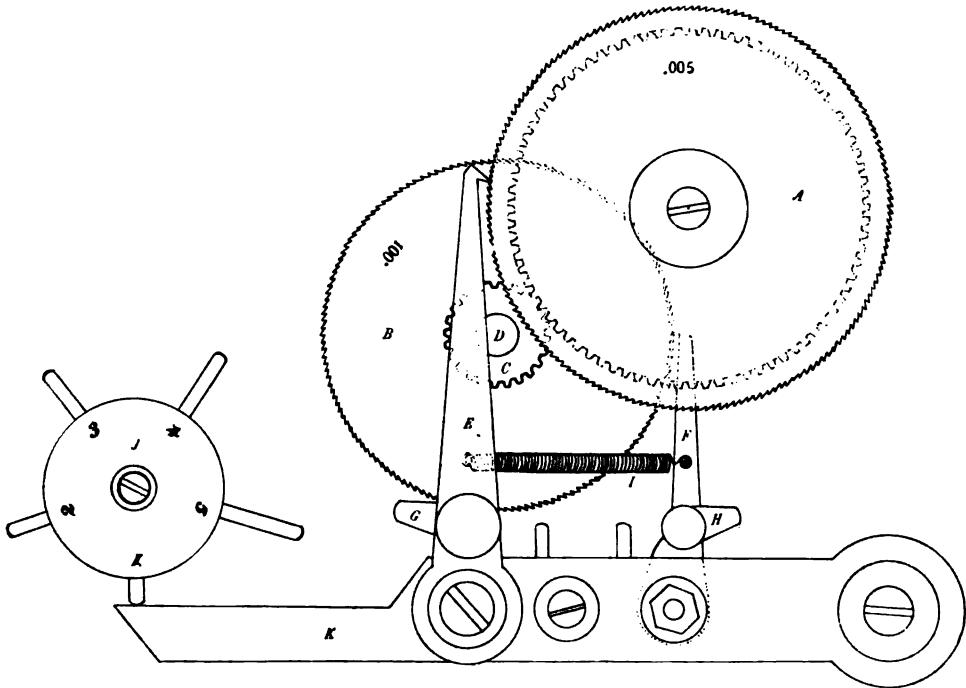


FIG. 1.

spindle D, on which the gear wheel C and ratchet B run, is eccentric on the shaft O. By means of the lever L this shaft is turned in its support N so that the gear may be readily thrown into or out of play. The shaft O may be held in any position by the thumb-screw M.

It is thus readily seen that this attachment is very little more complicated than that of the ordinary Minot, and is just as compact.

One objection is to be raised to it. One can cut sections ranging from 1 to 5 micra, but after that it must be 10, 15, 20, or 25  $\mu$ . That is, there is no intermediate thickness between 5 and 10  $\mu$ , etc. This might be obviated by making the notches of A smaller so that they would equal 3  $\mu$ , or even less. The author has not found the above objection a serious drawback, and believes it of little importance.

A photograph of the machine showing the attachment is seen in Fig. 4, which shows the general appearance.

A device for holding the microtome

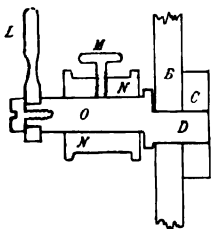


FIG. 2.

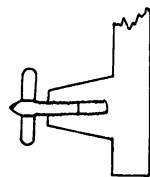


FIG. 3.

in position while cutting is shown in Fig. 3. It consists of adjustable screws with pointed ends screwed into each leg of the base. These may also serve as

FIG. 4.

leveling screws. With these pointed supports resting on the table the weight of the microtome is sufficient to hold it very firmly wherever it is placed.

University of Chicago.

JAMES ROLLIN SLONAKER.

### A Simple Method of Preparing Bone Sections.

The rough sections are cut with an ordinary butcher's bone saw, or small hack-saw. In a small block of soft pine, say 2 cm. wide, 3 cm. thick, and 4 cm. long, make a cavity of proper shape and depth for the section to fit in snugly. The block may then be conveniently held on a revolving grindstone, and wood and bone be ground down together. As soon as the first side is well smoothed, it is advisable to turn the section and grind the second side until near the desired thickness is reached. The section may now be removed and finished on an oilstone or hone, and finally mounted in thick balsam. Some care must be used, especially with longitudinal sections, to hold the block so that the grinding may proceed in the proper direction. The method may also be applied in making coarse sections of teeth, though less successfully than in working with bone.

H. G. ROSENBERGER.

Whitlin, Calif.

**LABORATORY PHOTOGRAPHY.***Devoted to Methods and Apparatus for Converting an Object into an Illustration.***A PHOTO-MICROGRAPHIC DEVICE.**

Mr. Frederic E. Ives, Franklin Institute, Philadelphia, Pa., has kindly submitted a model of his photo-micrographic camera for our examination. The following are the essential features :

In the construction of this photo-micrographic attachment, four conditions were constantly borne in mind, viz., that nothing should interfere in the slightest degree with the comfortable use of the microscope in the usual way ;

FIG. 1.—Camera adapted to Bausch & Lomb B B Microscope.

that the microscope might be used at any desired inclination or tube-length, and with any convenient source of light ; that the adaptation and removal of the camera should occupy very little time, and that the amplification and definition in the photograph should always correspond to that seen in the microscope.

The camera consists of a box having at the rear end any ordinary form of

FIG. 2.—Proboscis of Blow Fly. B. & L.  $\frac{7}{8}$  objective; Series I Huyghenian eye-piece.

plate-holder and provided at the front end with a fixed focus lens of ten inches focus, so that the amplification and definition of the image in the photograph will be exactly what would be calculated for the microscope image, and so that the definition will be equal to that seen in the microscope. A camera thus constructed, when applied to the eye-piece of the microscope at the eye-point, will reproduce the image seen in the microscope, correct in definition and amplification, provided the microscope has been focused with a normal eye and that the photograph is made by the action of the same light rays, which should be mono-

FIG. 3.—*Pleurosigma angulatum*, x 2375. Enlarged direct from negative.

chromatic, that form the visual image. If the microscope image is focused by a short-sighted or far-sighted eye, the focus should be obtained through a glass which corrects the eye for parallel rays, otherwise slight readjustment of the focus of the microscope may be necessary to make the image perfectly sharp on the ground glass of the camera.

The camera is mounted on a frame, supplied with rack and pinion, so that it may slide longitudinally to and from the microscope in alignment with its optical axis. The two side arms of this frame are pivoted to

brackets, securely attached to the baseboard of the apparatus in such manner that microscope and camera may be tilted to any desired inclination, from horizontal to vertical (Fig. 1).

The apparatus appears to be far less sensitive to vibrations when used at an angle of 45° than when either horizontal or vertical. Although this simple apparatus will not produce photographs of large size, such as can be made with projection eye pieces and very long cameras, nor include wide angles of view in low powers, such as may be obtained with Planar lenses without eye-pieces; photo-micrographs made with it, ranging from comparatively low amplification up to 1,500 diameters, are

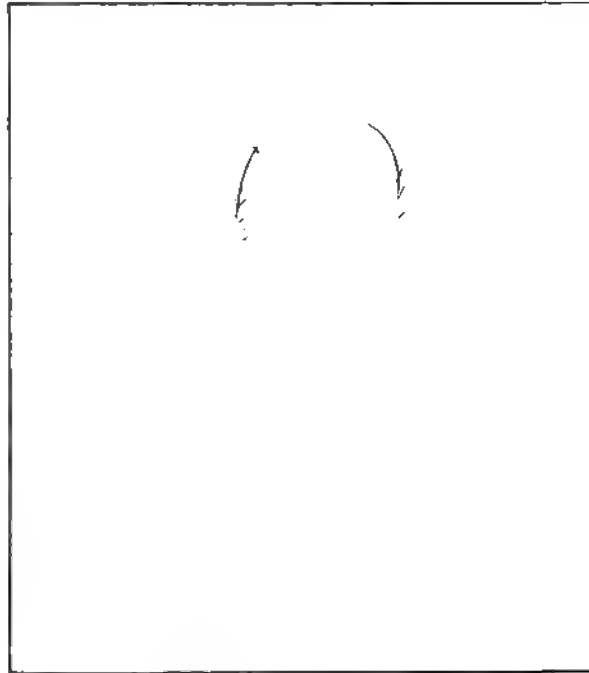


FIG. 4.—Antenna of Fly. B. & L.  $\frac{3}{8}$  objective; Series I Huyghenian eye-piece.

FIG. 5.—*Coscinodiscus asteromphalus*,  $\times 2000$ .

not inferior to those made with far more elaborate and expensive apparatus.

The advantages claimed for the apparatus may be summed up as follows : Weighs only three and one-half pounds. The microscope in ordinary use at any position or adjustment does not have to be moved or in any way disturbed when making a photograph. The camera is brought into alignment with axis of microscope by a single movement as a whole and without refocusing, or even looking at the image on the ground glass, the same definition and amplification as seen in the microscope can be obtained. The author states that it takes him from twenty to thirty seconds to attach the camera and adjust ready for an exposure. Is very inexpensive.

C. W. J.

### SOME OBSERVATIONS ON THE SEVENTEEN YEAR LOCUST.

The cicada photographed had been feeding lower down upon the same trunk, and was disturbed by the motions made in adjusting and focusing the camera so that it moved away from its then location and, after a time, readjusted itself in the one where it was photographed. When moving, the beak is folded back between the fore legs at an angle of about 45 degrees, so that the tip is just free from the surface of the bark. As it begins to feed, the tip of the beak is brought

FIG. 1.—Male locust feeding on the bark of a young peach tree.

forward so as to become more nearly perpendicular, and is rested against the bark. The insect then "backs up" so as to bring the upper end of the beak in perpendicular position above the tip, which is already resting against the bark. This backward movement on the part of the insect is accompanied by a setting of the legs in a firm position, and is accomplished by an actual pulling back of the body by the hind legs rather than a mere settling closer to the bark (Fig. 1). With the beak now in perpendicular position (see Fig. 1), the setæ apparently are protruded through the tube which the beak forms (see Fig. 2) and this is accompanied by a slight sinking of the head as the beak itself appears to be forced to a slight

degree into the bark. Having thus inserted the sucking apparatus, the cicada drinks its fill or until disturbed, and its source of supply is so abundant that when the beak is withdrawn more or less liquid follows the withdrawal of the beak, and affords an attraction for sap loving insects as shown in Fig. 3. The insect is so intent upon feeding that with care one can snip off the beak with slender scissors so that almost its full length will remain in the bark, as was

FIG. 2.—*a*, Beak of locust clipped off while insect was feeding on bark of apple tree.  $\times 10$ . *b*, Globule of sap.

done in preparing Fig. 2. This is not so easily done, however, as is the snipping off of the ovipositor in place, which, owing to the depth to which it is inserted in the wood, cannot be withdrawn so readily as is the slender and flexible beak. The insects seem to be sensitive to sudden motion rather than to near or strange objects in themselves, as difficulty in approaching the insects was almost entirely obviated when focusing cloth and other swinging or flapping articles were removed. But even when approached successfully, the feeding

FIG. 3.—Ants feeding upon juices flowing from a puncture in the bark of pear tree made by cicada.

cicadas were likely to move just at the wrong time on account of the movements of the camera body, which had the lens about 15 inches from the object in order to secure a slight magnification with extended bellows. In Fig. 2 the beak is shown raised a little from the bark, setæ show both above and below the beak. When cut, a globule of sap nearly as large as the pin head, shown at "b," formed on the cut end "a," but before it could be photographed the globule disappeared either by evaporation or by suction back into the wood.

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FREDERICK H. BLODGETT.

## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### IX. ABNORMAL CONSTITUENTS OF THE URINE—Continued.

#### ANATOMICAL SEDIMENTS.

*Blood.* Corpuscles in the urine present various appearances due to the action of the urine. In general they appear in small round discs requiring a one-sixth inch objective to recognize them. Some appear crenated with spinous projections, others as barely visible discs having lost their hæmaglobin. Urine containing blood is usually cloudy and varies from a dark red in acid urine to a bright red in alkaline urine to merely traces of color, depending upon the quantity present. Hæmaturia occurs in acute and chronic Bright's disease, nephritis, in calculus, and in malignant growths. Hæmorrhage from the bladder occurs in

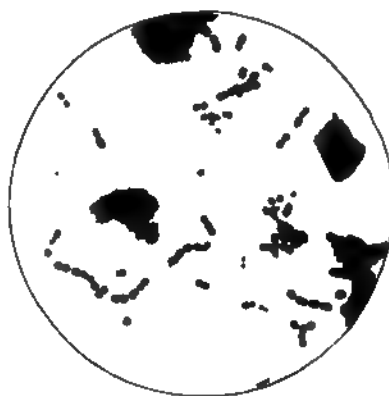


FIG. XIV.—Staphylococcus of Pus. Stained with Loeffler's alkaline methylen blue. Magnified 1200 diameters;  $\frac{1}{4}$  oil immersion objective, Zeiss projection ocular No. 4.

FIG. XV.—Streptococcus of Pus. Stain, methyl violet. Magnified 1800 diameters;  $\frac{1}{4}$  oil immersion objective; Zeiss projection ocular No. 4.

stone of the bladder, in cystitis, and in malignant growth. When hæmaturia is of urethral origin, the hæmorrhage precedes the flow of urine.

*Pus* is the most common of all of the anatomical elements. It is present in the simplest cases and in the most malignant. Pyuria is a constant symptom of acute and chronic nephritis, cystitis, prostatitis, etc. The pus cell is rounded and a little larger than the blood corpuscle. Add a little acetic acid to the sample, which will render visible the nuclei of pus corpuscles and differentiate them from blood.

*Epithelium* occurs in all urine and ordinarily has but little clinical significance. Epithelial scales from the bladder consist of flattened squamous cells, the deeper cells being of the transitional variety. Epithelium from the kidney may be columnar or irregular in form.

## TUBE CASTS.

Tube casts are probably the coagulable portion of the blood which passes into the uriniferous tubules, coagulates, and is then washed out by the urine. There are many kinds of casts, which should be carefully studied as they are of the greatest diagnostic significance.

FIG. XVI.—Hyaline cast. Sediment from centrifuge; slightly stained with carmine to photograph. Magnified 150 diameters;  $\frac{2}{3}$  objective, Zeiss projection ocular No. 4.

FIG. XVII.—Fatty cast. Treated same as Fig. XVI.

Hyaline casts are colorless, almost transparent structures. They are soluble in acetic acid, and in consequence should be examined from urine that has not been treated in any way. Fill the tubes of a centrifuge with urine and revolve them for three minutes. Decant the urine and transfer a drop of the sediment



FIG. XVIII.—Waxy Cast. Treated same as Fig. XVI.

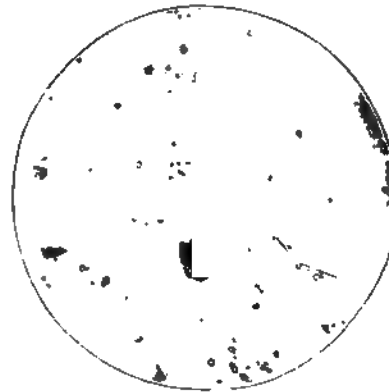


FIG. XIX.—Epithelial cast. Stained with hæmatoxylin. Magnified 150 diameters;  $\frac{2}{3}$  objective; Zeiss projection ocular No. 4.

to a clean slip. Lay a hair in the drop and apply a cover. Examine with a low power. Hyaline casts are very difficult to see as they are almost transparent. Reduce the light until the field is quite dark, and if possible, move the mirror to throw the light on the object obliquely. Casts are larger than any of the other elements in the urine and can hardly be misinterpreted.

Blood casts are diagnostic of renal hæmorrhage. They are similar to hyaline casts with blood corpuscles adherent to, and embedded in, the cast.

Fatty casts are indicative of "Large White Kidney." The refractive oil globules composing the casts render them easy to recognize.

Waxy casts are smooth in character and are found in many diseases of the kidneys, and are not characteristic of amyloid kidney as was formerly thought.

Epithelial casts are studded with epithelial cells, and when found in abundance in albuminous urine are diagnostic of Bright's disease, and in all cases indicate an inflammatory condition of the parenchyma of the kidney.

In general, when casts are small and few and principally hyaline, they signify a mild nephritis. The gravity of the disease increases as the casts increase in diameter and number.

Spermatozoa will be found in the urine of healthy adults after coitus, after epileptic seizures and occasionally in typhoid fever. Their constant presence in considerable numbers constitutes the disease, spermatorhea, and is the result of sexual excesses or masturbation.

Spermatozoa will be found in the sediment, as centrifuged, appearing as minute thread-like bodies with a head, body and tail. A high power (one-sixth inch objective) will be required to show them distinctly.

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## METHODS IN PLANT PHYSIOLOGY.

### IV.

#### NUTRITION.

1. **To Determine the Elements Necessary for the Nutrition of Plants.** The dependence of plants upon certain elements for nutrition may best be demonstrated by supplying plants with culture solutions of known chemical composition. The more important facts may be brought out by planting seeds in clean sand and watering the jars with the culture solutions, but the best results are obtained by the use of water cultures. The method of preparing the cultures, which is essentially that given by Sachs<sup>1</sup>, is as follows: Wash thoroughly a number of pint fruit jars, using dilute hydrochloric acid and rinsing well with distilled water, finally fill each jar to within 2 cm. of the top with distilled water. Weigh out the salts for each jar according to the following formula, which contains all the elements necessary for growth, make up a series of solutions, each one of which shall lack one of the necessary elements, e. g., if potassium is to be omitted substitute calcium nitrate for potassium nitrate, etc. Make one or two full solutions for the sake of comparison, omitting no chemicals:

Potassium nitrate,	-	-	-	-	-	.5	gram.
Sodium chlorid,	-	-	-	-	-	.25	"
Calcium sulphate,	-	-	-	-	-	.25	"
Magnesium sulphate,	-	-	-	-	-	.25	"
Calcium phosphate,	-	-	-	-	-	.25	"
Iron chlorid, aqueous solution,	-	-	-	-	-	3-4	drops.

<sup>1</sup> Lectures on the Physiology of Plants, p. 283.

The sodium chlorid should be included to keep the solution alkaline, it serves as a preservative, not as a plant food. The iron is not used as a food, but is necessary for the production of chlorophyll; if at any time the plants in the cultures begin to appear etiolated, a few drops of iron chlorid solution should be added to the jars. New, clean corks are now fitted to the jars, using a sharp knife and a coarse file to trim them; two holes should be cut in each cork, one for the plant and one for a glass tube to admit air. The tube should project about 7 cm. on either side of the cork, it also provides a means for introducing fresh water when necessary. The hole for the plant should be at the center of the cork and about 1 cm. in diameter; the cork is now evenly divided through the center and placed in the mouth of the jar. Sterilize the jars and their contents by boiling them in a water-bath for thirty minutes, or by placing them in a steam sterilizer and subjecting them to hot steam for forty minutes. As soon as removed from the sterilizer add distilled water, if necessary, to replenish the loss from evaporation during sterilization, plug the end of the glass tube and the hole in the cork with cotton, and allow the jars to cool.

Seedlings of corn (*Zea Mais*) or of buckwheat (*Fagopyrum esculentum*) are well suited for use in water cultures. Select seedlings 3–6 cm. long which have grown in damp sawdust; all adhering particles of sawdust must be removed by means of a camel's-hair brush and distilled water, finally they should be rinsed with two or three changes of distilled water. When the temperature of the jars is between 18° and 25°C. the seedlings may be placed in them. Wrap

the seedling with a tuft of fresh, dry cotton-batting, remove half of the cork and place the seedling in the semi-circular hole at the center so that the collar of cotton-batting supports the seedling when the other half of the cork is replaced. The cotton should never be allowed to become wet, as it is pretty sure to produce a growth of fungus; on that account freshly ignited asbestos is better than cotton. In the case of the corn seedling the grain should go below the cork, but should not touch the liquid, the upper end of the corn or buckwheat seedling should project above the cork. The interior of the jar must be rendered dark to prevent the growth of algæ in the solution; for this purpose cover the jar with an opaque jacket (Fig. 4). When finished, label the preparations indicating the composition of the solution and place the cultures in a hot-house. At the end of two weeks, and each week thereafter, empty the jars and refill them with fresh solutions of similar chemical composition; from time to time ærate the solutions by forcing air in through the tube. Final observations may be made at the end of three or four weeks.

2. **The Acidity of Roots.** The presence of acid in the secretion of the root may be shown by its reaction to litmus paper. Grow any seedlings with a large

FIG. 4 — Apparatus for determining the elements necessary for the nutrition of plants.

primary root in a damp chamber until they have attained a length of 5–6 cm. Moisten a piece of blue litmus paper with distilled water and bring it into contact with the terminal 2 cm. of the root. A decided acid reaction will be shown.

3. **The Corroding Action of Roots.** Another method of demonstrating the corrosive action of roots is by allowing them to grow against marble. Embed a piece of marble with a polished surface about 6 cm. by 10 cm. in the bottom of a crock of sand, incline the polished surface so that the roots growing downward will strike upon it. If the surface of the block shows scratches they should be sketched at the time of making the preparation, and the sketch preserved for reference at the final observation. Plant above the marble seeds of the bean (*Phaseolus communis*), watermelon (*Citrullus vulgaris*), or *Vicia Faba*; when the stems are 2–4 cm. above the surface of the sand, empty out the contents of the jar and examine the corrosion figures on the marble.

4. **Nutrition of Fungi.** The elements necessary for the nutrition of fungi may be determined experimentally in the same way as in the case of chlorophyll-bearing plants. It will be noted that the following formula contains all the elements used for the water cultures, with the addition of two organic substances—dextrose and peptone—though not all fungi need both dextrose and peptone. It is more difficult to obtain satisfactory results with cultures of fungi than with those of green plants for two reasons—a slight impurity in the culture solution is not so quickly exhausted as in the cultures of the larger green plants, and many fungi can make considerable though not full growth without the presence of all the mineral substances. Carefully clean and dry a number of 200 c. c. Erlenmeyer flasks; in each of two flasks place half of the following formula, with such modifications as are necessary to omit one element in turn from each pair of duplicates:

Dextrose,	-	-	-	-	-	-	5 grams.
Peptone,	-	-	-	-	-	-	1 gram.
Ammonium nitrate,	-	-	-	-	-	-	1 "
Magnesium sulphate, crystals,	-	-	-	-	-	-	.25 "
Potassium monophosphate,	-	-	-	-	-	-	.25 "
Calcium chlorid,	-	-	-	-	-	-	.01 "
Distilled water,	-	-	-	-	-	-	100 c. c.

The flasks are then closed with firm plugs of cotton-batting, and sterilized in hot steam for 40 to 50 minutes on each of three successive days. When the flasks are cool the culture medium in each is to be inoculated with the spores of *Penicillium glaucum*, or of *Phycomyces nitens*; then labelled, stating what was omitted from the culture fluid. Observations should be made each week for three or four weeks.

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## A Review of the Methods of Staining Blood.

The vast amount of work on the blood in recent years, consequent to its growing importance in diagnosis and to the activity in the investigation of the malarial parasites, has produced such an extensive literature and has developed such a complexity of methods for preparing and staining this aggregation of cells for microscopical study that a collection of this literature and a review of these methods seem worth the while. For the earlier literature on this subject I am indebted to Müller, *Die Methoden der Blutuntersuchungen, Zusammenfassendes Referat* (Centralbl. f. allg. Pathol. u. path. Anat., 1892, Oct. 31, Nov. 18), and to Mannaberg, *Die Malaria Parasiten*, Wien, 1893. The introduction of dry blood films in the study of the blood by Ehrlich in 1878-79 supplied a simple and quick method of preparing the blood cells for staining which has superseded all others. And nearly all of the subsequent literature on staining the blood has been concerned with these dry films. For these reasons this review will be occupied chiefly with the methods of preparing, fixing and staining dry blood films.

### I. MAKING THE PREPARATIONS.

Blood from the human subject is best obtained by puncturing the thoroughly cleansed lobe of the ear or the tip of the finger with a three-cornered surgeon's needle, from most other mammals by puncturing one of the small veins of the ear, and from the lower vertebrates and some of the small mammals by chloroforming the animal and taking the blood from the internal organs. In every case one should endeavor to obtain small, freshly exuded drops rather than an extensive flow of blood.

The dry blood film consists essentially of a small drop of fresh, uncoagulated blood spread upon a cover-glass, one layer of corpuscles deep, which dries almost instantly, fixing the corpuscles flat upon the glass before they have time to shrivel or become distorted. The main points in the somewhat difficult technique are cover-glasses absolutely clean and free from greasiness, the use of only a small drop of freshly exuded blood and rapidity of manipulation. Two general methods for securing this thin distribution of the blood upon the cover-glass are in use, some preferring one and some the other method.

1. **The Method of Ehrlich.**—This is the method more generally recommended. It consists in touching the surface of a cover-glass to a small drop of fresh blood and dropping it blood downward upon a second cover-glass. The blood spreads by capillarity in a thin layer between the two cover-glasses, which are immediately slid from off one another without lifting apart. If the cover-glasses were clean and free from grease and the proper amount of blood was taken a thin film of blood is left on both cover-glasses, which are placed blood upward to dry. The sliding apart of the cover-glasses is facilitated by dropping the first upon the second so that their corners do not coincide. The drying of the blood films can be hastened by waving the cover-glass to and fro in the air.

**A Modification of the Method of Ehrlich.**—Several pairs of cover-glasses placed

face to face are arranged conveniently before the operator. A pair of these is picked up with forceps and an edge of them applied to the drop of blood. The blood flows by capillarity between the two cover-glasses, which are then withdrawn from the drop of blood, slid apart and turned up to dry as in the Ehrlich method. The advantage of this method over the original method of Ehrlich is the ability to gauge the amount of blood that is needed. By the method of Ehrlich the amount of blood taken depends upon the size of the drop and the judgment of the manipulator; by this method when the film of blood has spread throughout between the two cover-glasses they are withdrawn from the drop of blood and the supply instantly cut off. By this method preparations can also be made from more extensive flows of blood as are often unavoidably obtained from the internal organs of the lower vertebrates.

2. **The Method of Smith, Mannaberg, and Others.**—By this method a square cover-glass, preferably held in a pair of clamp forceps, is touched at one corner to the drop of blood and the edge applied to the surface of a second cover-glass. The blood at the corner immediately flows along the whole line of contact, and by drawing the first cover-glass held at an angle of  $45^{\circ}$  across the surface of the second cover-glass, a thin film of blood is left on the latter. The use of the end of a glass slide instead of the edge of a cover-glass is recommended by several as a more convenient implement for spreading the blood.

In my own experience preparations can be made more rapidly, with less regard to the quantity of blood taken and thinner by this method than by the method of Ehrlich; all of which are important advantages in the technique. Preparations can also be made by this method from extensive flows of blood and from the bone marrow.

*Horder* (1899) recommends a piece of gutta-percha half an inch square for receiving and spreading the blood. The gutta-percha is held in one pair of Ehrlich forceps and a cover-glass in another pair. The blood, as it exudes in a fresh drop, is touched by the edge of the gutta-percha, which is laid flat upon and drawn across the cover-glass, commencing from the edge held by the forceps. Cover after cover can be spread as rapidly as they can be picked up with the forceps.

## II. FIXING THE PREPARATIONS.

As the term "fixation" is generally employed in histological technique it refers to the killing and preserving of the cytological elements and their structure, especially the finer structure of the nuclei, for microscopical study. In dry blood films, fixation in this sense of the word is accomplished in the process of rapid drying, and any subsequent treatment of the preparation has little effect on the preservation of the cell structure. Various methods other than rapid drying have, however, been recommended for killing and preserving the cytological elements of the blood.

### A. *Fixing Fresh Blood.*

1. **Alcohol.**—Bizzozero (1882) and Cornil (1887) put the fresh, undried blood films in absolute alcohol for one hour or more.

2. **Alcohol, Ether, and Sublimate.**—Gulland (1887) recommends the following mixture for fixing undried blood films :

Absolute alcohol saturated with eosin,	-	25 c. c.
Pure ether,	- - - - -	25 c. c.
Sublimate in absolute alcohol (2 g. to 10 c. c.)		5 drops

The fresh preparations are dropped wet side downward into the fixing solution. The fixation is practically instantaneous, but the preparations should remain in the solution at least three or four minutes to fix the film to the cover. The preparations are stained by the eosin in the fixing solution and may be counterstained with methylen blue.

3. **Chromic Acid.**—Harris (1883) used chromic acid ( $\frac{1}{8}$  per cent.) and also potassic bichromate ( $\frac{1}{12}$  to  $\frac{1}{2}$  per cent.); preparations to remain in the fixative several days, then wash twelve to twenty-four hours in running water.

4. **Flemming's Solution**—Cornil (1887) fixed fresh blood films in Flemming's solution for several hours.

*Muir* (1891) dropped the fresh blood into strong Flemming's solution, embedded the drop in paraffin and sectioned. "This method shows the structure of the leucocytes well, but the character of the red corpuscles is poorly shown, and in ordinary conditions but few leucocytes are seen in one section."

*Deetjen* (1901) used Flemming's solution for fixing blood plates in fresh blood spread on an agar solution instead of glass (see IV, 9). The fixative was allowed to flow under the cover-glass for from three to five minutes.

5. **Formalin.**—Heiman (1898) recommends formalin,—

Formalin (40 per cent.)	- - - - -	10 c. c.
Water,	- - - - -	30 c. c.

Fresh, undried blood films are exposed to the vapor of this solution contained in a wide mouth bottle for from five to ten minutes. The solution will remain good about two months.

*Kiser* (1900) gives the following method of fixing blood with formalin: "Mix one volume of perfectly fresh blood with three volumes of a two per cent. solution of formalin. Allow the mixture to stand at least an hour; then draw a small quantity from the bottom of the vessel with a pipette, by which a drop is transferred to a clean coverslip, and allow the liquid to evaporate. The method of pressing the coverslips together, as in sputum analysis, is to be preferred. Pass the coverslip through the flame, film uppermost, in order to cement the corpuscles to the glass. Dip into a five per cent. solution of acetic acid once or twice, and remove the acid with water."

6. **Hermann's Fluid.**—Müller (1892) used Hermann's fluid (1 per cent. platinic chloride 15, 2 per cent. osmic acid 4, acetic acid 1). The preparations, either absolutely fresh or more or less dried, were brought into the fluid and allowed to remain several days to a month (Müller recommends ten days). Wash in running water twelve to twenty-four hours.

7. **Mercuric Chloride.**—Freeborn (1889) put a drop of mercuric chloride in the form of Pacini's or Hayem's solution on the finger and pricked the latter through the fixative. The drop of fluid and the drop of blood were mixed with the needle, and then transferred to a slide and covered.

*Muir* (1891) made the Ehrlich films and placed them at once, before any drying occurred, on the surface of a saturated solution of corrosive sublimate to which was added  $\frac{3}{4}$  per cent. of sodic chloride,—preferably heated to a temperature of 50° C.—where they were allowed to remain for about half an hour. The preparations were then thoroughly washed in  $\frac{3}{4}$  per cent. common salt solution, taken through successive strengths of alcohol and then stained. It is recommended that salt in the same proportion be added to the weaker strengths of alcohol.

*Römer* (1892) placed the fresh blood films directly into a saturated watery solution of sublimate for six minutes, then into distilled water for two minutes, absolute alcohol one minute, distilled water one minute, followed by the staining solution—in this case hæmatoxylin followed by eosin.

8. **Osmic Acid.**—*Afanassiew* (1884), *Hayem* (1890), *Laveran* (1891), *Luzet* (1891), and *Heiman* (1898) exposed the undried blood films to the vapor of osmic acid. *Heiman* used 1 per cent. solution. *Laveran* mixed a drop of blood with a drop of a solution of osmic acid 1 in 300 on a slide, applied a cover-glass, and permitted the stain (glycerin in which picro-carmin is dissolved) to flow through. This method was used for malarial blood.

*Deetjen* (1901) used the vapors of osmic acid or one per cent. osmic acid to fix blood plates in fresh blood in which the cells were kept from dying by being spread on his agar solution (IV, 9).

9. **Osmic Acid and Acetic Acid.**—*Dekhuyzen* (1901) used 3-1 or 9-1 osmic acid (3 or 9 volumes of 2 per cent. osmic acid with one volume of 6 per cent. acetic acid, containing  $\frac{1}{4}$  per cent. methylen blue) for fixing blood plates. With invertebrates the acetic acid sometimes produces an objectionable precipitate of granular albumen, then osmic acid was used alone.

10. **Picric Acid.**—*Freeborn* (1889) gives *Gage's* method for amphibians' blood: "Three or four drops of fresh blood are allowed to fall into 10 c. c. of normal salt solution, contained in a tall glass cylinder. Agitate thoroughly, and mix with 100 c. c. of a saturated aqueous solution of picric acid with constant stirring. Allow the blood cells to settle, and pour off as much of the supernatant fluid as possible, add an equal amount of normal salt solution; continue this until the salt solution is only slightly tinged yellow. Then add 10 c. c. of a mixture of 5 parts of carmin and 95 parts of picro-carmin for staining. This requires about 15 hours. Then pour off as much of the staining fluid as possible and add 10 c. c. of acid glycerine (glycerine 100 c. c., hydric or formic acid 1 c. c.). The cells may be kept in this mixture indefinitely. For mounting, remove a drop with a pipette, place it on a slide, cover, and cement the cover immediately.

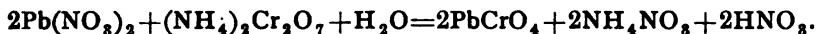
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## MICRO-CHEMICAL ANALYSIS.

## XX.

## SILVER GROUP CONTINUED—LEAD.

*IV. Ammonium Dichromate added to solutions of salts of Lead precipitates Lead Chromate.*

*Method.* To the drop of the solution to be tested add a drop of dilute nitric acid. Next to the test drop thus prepared place a drop of a solution of the reagent. Heat the two drops simultaneously and while they are hot cause the reagent to flow into the test drop. Thin, slender, yellow prisms (monoclinic?) are immediately formed (Fig. 83). These prisms are sometimes grouped in radiating masses. More often they occur singly.

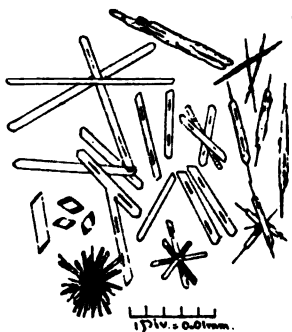


FIG. 83.

*Remarks.* The addition of the reagent to a cold neutral solution seldom yields more than a heavy amorphous precipitate bright yellow in color.

From barium chromate the lead salt is readily distinguished by adding a minute amount of sodium or potassium hydroxide; under this treatment lead chromate is converted into basic chromate, orange red or even bright red in color of variable composition. Too much alkali will completely dissolve the lead chromate; but if to this alkaline solution ammonium carbonate is added, red basic chromates are precipitated.

Mercurous salts when treated as described above yield bright red crystals of peculiar form and variable composition (see Mercury). Although it is not likely that these salts can be confused with the lead compound, if any doubt should arise, they can be differentiated by treating with ammonium hydroxide, in which the mercurous salts are not soluble while the lead compound dissolves.

The behavior of mixtures of silver and lead has already been described under the former element. Lead does not form a dichromate. In the presence of silver interesting mixed chromates of silver and lead separate.

Potassium chromate yields results similar to those obtained with the dichromate.

The chromates of strontium and barium have already been discussed under those elements.

Thallous salts yield with ammonium dichromate long acicular crystals of thallous dichromate  $\text{Tl}_2\text{Cr}_2\text{O}_7$ .

*Exercises for Practice.*

See suggestions given under Mercury.

E. M. CHAMOT.

## The Technique of Biological Projection and Anesthesia of Animals.

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### VII. PROJECTION MICROSCOPES USING ELECTRIC ARC OR OXYHYDROGEN LIGHT.—Continued.

Higher power objectives are to be tested in the same manner after the sub-stage condenser has been put into place. In working with high power objectives, it is necessary to keep in mind that the fields of such lenses are small, being only  $\frac{1}{16}$  of an inch in diameter in a  $\frac{1}{4}$ -inch oil-immersion, and that this small field must be perfectly illuminated for successful work. It is readily seen by observing an arc for a time through smoked glass, or a combination of two plates of deep ruby and blue glass, that the arc creeps about on the ends of the carbons as they are slowly consumed. This variation in the position of the luminous point carries it away from the optical axis at the lamp and shifts it a corresponding distance on the opposite side of the axis at the object. There are three ways for overcoming this difficulty. First, by using soft-cored carbons; second, by using as small a horizontal carbon as possible in combination with a larger vertical carbon; and third, by moving the luminous point into the optical axis by a slight vertical or lateral movement of the arc. To accomplish the last, the vertical supporting rod of the lamp should not be clamped rigidly and a slight lateral push on the feed-wheels will rotate the lamp on its support. Small carbons burn away more rapidly than large ones and the feed requires more frequent attention. The carbons shown in the engraving are  $\frac{7}{16}$  and  $\frac{9}{16}$  inches in diameter, "Nurnberg soft-cored, electra brand." In the highest power work which the writer has done with the electric light, a very steady light was obtained by using a  $\frac{9}{16}$ -inch cored vertical and a  $\frac{5}{16}$ -inch solid horizontal carbon. The light from a 110 volt alternating incandescent current was sufficient to give a strong picture of a stained transverse section of an earthworm, having a magnification of 8800 diameters by measurement. The objective used was a B. & L.  $\frac{1}{4}$ -inch oil-immersion in connection with an amplifier.

The conditions attending the production of the best light, as described above, indicate the reason for the use of hand-feed 90° arc lamp rather than any of the automatic-feed lamps or other types of hand-feed lamps. With the 90° hand-feed lamp the arc is under the control of the operator at every instant, while with the automatic-feed the operator is frequently under the control of the feeding mechanism at the very instant when his live specimen is at its best. The automatic-feed lamp is convenient in very low power and lantern slide projection, but here also the 90° lamp gives as good results.

Concerning the field of illumination on the screen it should be noted that, if the microscope is pushed too close to the lamp, the field will be blue and will not give satisfactory results. As the microscope is moved to a greater distance from the lamp, the center of the field will be strongly illuminated, and at a still greater distance the entire field will be evenly illuminated, and this is the best

position for all objects except the most difficult, which may require the strong central illumination.

The system of condensers is of the utmost importance. The best combination for microscopic projection consists of three plano-convex condensers, each four and one-half inches in diameter, and arranged as follows: No. 1 is double thick lens of about five inches focal length with its plane side next to the light; No. 2 is a medium thick lens of six and a half inches focal length with its convex side facing the convex side of No. 1, and enclosed in the same cell with it; No. 3 is a thin lens of about eleven inches focal length, and is attached to the water tank with its convex side facing the microscope.

A simple plano-convex sub-stage condenser of  $\frac{1}{8}$  inches focal length is a necessity in high power work with the above named condensers, but its efficiency varies with its distance from the object. The correct distance for each objective should be determined by experiment and recorded for reference. Begin the test with the sub-stage condenser pushed up close to the object and slowly draw it away.

All the directions given for centering, adjusting and testing the working distances with the electric lamp apply equally well to the oxyhydrogen light, in which the luminous point is constant, but the less intense light materially reduces the maximum magnification.

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University of Chicago.

## LABORATORY OUTLINES.

For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

### A SERIES OF FORMS TO ILLUSTRATE THE EVOLUTION OF SEX.

#### XVI. *Eudorina elegans*, Ehrb.

Order, Protococcales. Family, Volvocaceæ.

*Eudorina* frequently occurs in pools of rain water, in ponds, and in marshes. The colonies are hollow, free-swimming bodies, more or less spherical in shape, usually consisting of thirty-two cells which are considerably separated from each other.

1. Mount a drop of water containing the organism and examine under low power. Under high power draw a single colony, showing the arrangement of the cells.

2. Draw a single cell, showing the two flagella, the red eyespot, and the chloroplast with a pyrenoid.

3. Vegetative propagation. The individual cells divide into sixteen or thirty-two new cells, and these escape as daughter colonies the same as in *Pandorina*. Draw a colony showing daughter colonies, and describe.

4. Sexual reproduction. The colonies are either unisexual or hermaphrodite. Draw a colony showing antherida (spermaries), consisting when mature of plates

of sixty-four small cells each, which develop into male gametes (spermatozoids). Draw and describe free-swimming spermatozoids.

5. Draw a colony containing female gametes (oospheres). The colony with oospheres differs very little from the ordinary vegetative colony. Watch for spermatozoids swarming about the female colonies.

6. Draw and describe the ripe, red colored oospore.

7. NOTE.—*Eudorina* shows a considerable advance in sexual development over *Pandorina*. The female gamete (oosphere) has become stationary, but still retains its flagella at first, and does not divide. The male gametes (spermatozoids) are formed by the repeated division of the cells of the colony. They are very small in comparison with the female cell, swim about freely in the water, and have lost their chlorophyll.

XVII. *Volox globator* L. Order, Protococcales. Family, Volvocaceæ.

This alga is of such size that its spherical, free-swimming body can easily be seen with the naked eye. In summer or autumn it can frequently be found in fresh water ponds and lakes.

1. Take up some of the spherical colonies with a large-mouthed medicine dropper or a glass tube and, having formed a little chamber on the slide with a xylonite ring or with paraffin, mount and study under low power. Note the rotating movements of the hollow, spherical organism.

2. Draw a colony showing the numerous cells and some daughter colonies, which appear as darker green spherical masses of various sizes.

3. Under high power, study a single colony. About how many cells in a colony of average size? Draw a few cells, showing the cell walls, the protoplasmic strands connecting the cells (protoplasmic continuity), the chloroplast, the red eye spot, the pulsating vacuole, and the two flagella of each cell. The flagella will be more distinct after staining with iodine.

4. Describe the development of a daughter colony from one of the cells of the mother colony. Look for an opening (the pore) in one side of the young colonies.

5. Sexual reproduction. The colonies are hermaphrodite, developing both sexual organs—the oogonia and antheridia—in late summer or autumn. Draw an antheridium. This represents an enlarged cell of the colony which has divided into a large number of elongated cells arranged like a bundle of asparagus shoots.

6. Draw an oogonium, projecting into the cavity of the colony, showing the enlarged oosphere. Draw a ripe oospore, showing the thick wall with peculiar angular spines on the surface.

7. NOTE.—In *Volvox* complete sexuality has been attained with the normal conditions of the sexual cells (gametes). It will be noticed that the plant is hermaphrodite, and this is the more usual condition in all but the higher plants.

Ohio State University.

JOHN H. SCHAFFNER.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Strasburger, Ed. Ueber Befruchtung. Bot. Zeitung. 59: 1-8, 1901. It will be remembered that Strasburger

in his paper on double fertilization<sup>1</sup> insisted that in fertilization two processes should be recognized: the stimulation to development and the mingling of ancestral qualities, the latter process being the essential one, and the stimulation to development only providing the conditions which make it possible to attain the advantages which result from a mingling of ancestral plasma masses.

Fluctuating variations do not furnish a starting point for the formation of new species. It is the principal function of fertilization, through the mingling of ancestral plasma masses, to keep the species characters constant. This view agrees with that of Richard Hertwig and approaches that of Solms Laubach in so far as the latter regards "so-called fertilization," or the fusion of hereditary masses, as an essential element in fertilization, but Solms Laubach regards the stimulation to development as an equally important attribute of fertilization. Strasburger, however, defines the so-called fertilization definitely as the union of the two hereditary plasma masses and believes that it was to insure this essentially "generative fertilization" that in the course of phylogenetic development the inability of the sexual cells to develop independently without fusion became more and more marked. The term "generative fertilization" is used to designate a union of ancestral plasmas in contrast with "vegetative fertilization," which is merely a stimulus to development. Were it not for the fact that the two processes occur simultaneously, the distinction would have been recognized long ago. The term, fertilization, has often been loosely used in cases like many nuclear fusions in fungi, where there is no union of hereditary masses but only a stimulus to development. While Strasburger would be willing to admit that the stimulus to development might be due to chemical or physical influences, he insists that "generative fertilization" is not a purely chemical process.

Winkler suggests that bastards might be produced by chemical fertilization. Strasburger regards this as impossible, and believes that the essence of fertilization lies in the union of organized elements.

The view that the epigenesis of form is only an expression of the epigenesis of chemical power, might, perhaps, appeal more to the physiologist than to the morphologist, who has studied more deeply into the developmental history of organisms. No doubt morphologists busied themselves too long with a one-sided, mechanical view of ontogeny; if the chemical theory does not in the same way go beyond the mark, the two views, united, should be useful in extending our knowledge.

C. J. C.

<sup>1</sup> Strasburger, Ed. Einige Bemerkungen zur Frage nach der Doppelten. Befruchtung bei den Angiospermen. Bot. Zeitung. 58: 293-316, 1900.

Osterhout, W. J. V. Cell Studies. I. Spindle formation in *Agave*. Proceedings of the California Acad. of Sciences. Ser. III. 2: 255-284, pls. 25-28, 1902.

In this work considerable attention was given to technique, especially to fixing. About forty fixing agents were tested by watching their effect upon the liv-

ing cell, and it was found that some of the most highly recommended solutions produce profound disturbances in the cytoplasm. For most objects fixing agents were found which produced no visible change in the living cell, as far as could be observed with a 2 mm. oil immersion lens during the application of the reagent, but even in some such cases the material undergoes structural changes after a few hours and the time must be shortened accordingly. Flemming's strong mixture proved to be the best in most cases; very fair results were obtained with iridium chloride, platinum chloride, palladium chloride, and Flemming's strong mixture with an excess of chromic. Material was washed from two to eight hours in running water and then dehydrated. Mixtures of absolute alcohol and bergamot oil were used for clearing.

Gentian violet was used for staining kinoplasm. If the stain washes out too readily, some gentian violet should be dissolved in the absolute alcohol. From two to twenty seconds immersion in dilute iodide iodine solution just before transferring to the absolute alcohol, often gives a sharper differentiation.

The summary of Prof. Osterhout's work on spindle formation in *Agave americana* is partly as follows: During early stages in the first division of the pollen mother cell the spindle is enclosed in a special membrane of cytoplasmic origin, which forms a complete investment around it. The functions of the membrane appear to be comparable to those of the nuclear wall and the limiting membrane of the cytoplasm. There is no weft of fibers such as is usually described for that stage. The spindle-forming fibers are radial from the beginning, and are attached both to the nuclear and the spindle wall. The second division differs radically from the first, the spindle-formation resembling in general that described for the spore mother cells of *Equisetum*.

The plates are in three colors, reproducing the effect of the safranin-gentian violet-orange stain.

C. J. C.

Leavitt, Robert G. Outlines of Botany for the High School Laboratory and Class-room (Based on Gray's Lesson in Botany.) 8vo. pp. 272, figs. 384, New York American Book Co., 1901.

This book was prepared at the request of the botanical department of Harvard University. The descriptive text follows, in the main, the sequence of

topics of Gray's Lessons in Botany and much of the phraseology is retained. More attention has been given to Cryptogams as well as to physiology and the relation of plants to their surroundings. The exercises and experiments are so chosen that schools with compound microscopes and convenient apparatus will have an opportunity to use their equipment, while, on the other hand, the needs of schools with only simple microscopes and limited equipment have been kept in mind.

The writer has succeeded in the task imposed upon him, but, in our opinion, the times have so far outgrown Gray's Lessons that a revision is impossible. Gray was the most progressive American botanist of his time, and were he still alive, we believe he would, like Sachs, refuse to make another revision of a book which needed to be replaced. We believe that Prof. Leavitt could have written a better book, had he not been handicapped by the old lessons.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Von Wendt, Georg.** Eine Methode der Herstellung mikroskopischer Präparate, welche für mikrophotographische Zwecke geeignet sind.

The author uses the following method as giving the best results: 1. Fixation and hardening. Material is cut in

3 mm. blocks, fixed in cold 3 per cent. nitric acid for 12–20 hours; nitric acid, alcohol and picric acid are also suitable. The block is carried direct into 90 per cent. alcohol for at least twenty-four hours. 2. Preparation for the first mordant. Blocks are put into alcohol (75 per cent.) 10 pts., ammonia 1 pt., for 6–10 hours at a temperature of 15°C., and then again into 90 per cent. alcohol for twenty-four hours. Then the tissue goes into alcohol (75 per cent.) 12 pts., HCl 1 pt., for 4–6 hours. After this it again goes into 90 per cent. alcohol for twenty-four hours. 3. Mordant (A) 5 per cent. ammonium-wolframate or ammonium molybdate solution. The so-called mordant is used for twenty-four hours, first at a temperature of 17°–20° C., and for the last few hours, 12°–15°C.; washing out in cold water and passing into 90 per cent. alcohol follows. 4. Imbedding. The usual paraffin method. 5. Cutting and fastening to slide. Sections must not be too thick; they are spread over warm alcohol, not water, and fastened to slide or cover with Mayer's glycerine—albumin. Paraffin is removed by xylol and slides carried as rapidly as possible to cold water. 6. Mordant B. The water is drained off and a 2 per cent. iron-alum solution dropped on to cover the sections. The slides are put for 2–7 minutes at a temperature of 55° C, the thicker the sections the longer the time. Wash in cold water. 7. Stain. A saturate solution of hematoxylin in alcohol is dropped into distilled water till the mixture has a moderately bright brown-gold color. This must stand a long time before using. This is dropped on the slide in the same way as the iron-alum solution, and allowed to act for ten minutes at 55° C. 8. Differentiation. These are differentiated in cold iron-alum till the desired tone is obtained. 9. Mounting. Washing in water and carrying to balsam by the usual method.

A. M. C.

**Floresco, N.** Correlation of Coloring in Liver, Skin and Hair. *Comptes Rendus*, 133 : 828–830, 1901.

Previous work by this author has shown that a snail with a dark shell has a dark mantle and dark liver; that a snail with a yellow-greyish shell has an

almost transparent mantle and yellowish liver. There is more iron in the livers and mantles of snails with dark shells than of those with light shells. These observations have been extended to dogs and cats, with the result that the liver and skin of animals with dark hair contain almost twice as much iron and pigment as those with light hair.

A. M. C.

**Gautier, Armand.** Chemical Basis of Variation. *Comptes Rendus*, 133 : 570-572, 1901.

of the cells, and this makes a sudden appearance of a variation possible without intermediate steps. A plant or animal organism may exhibit important variations with some abruptness; these variations have their basis in plasmatic molecular changes of particular organs. These plasmatic changes are referable to reciprocal influences of other plasmas and to a less extent to changes in environment and nutrition.

A. M. C.

**Dean, Bashford.** Reminiscence of Holoblastic Cleavage in Ovum of Cestracion. *Annot. Japon.* 4 : 35-41, 1 pl., 1901.

The interesting fact is noted that in the eggs of *Heterodontus* (*Cestracion*) japonicus, the uppermost surface, the animal pole, is traversed by definite, sharply marked lines, separating areas resembling the blastomeres in a late cleavage stage of *Lepidosteus*. The red-colored germinal disc is further down the side of the egg. The author argues that these lines are reminiscent of holoblastic cleavage. It can thus be concluded that the great size of shark eggs was attained before that cleavage was lost and that the yolk region of such eggs is homologous with the lower pole-cells in other Ichthyopsids.

A. M. C.

**London, E. S.** Examination of Hairs for Medico-legal Purposes. *Archiv. Sci. biologique St. Petersb.* 7 : 136-157, 6 pls., 1900.

The author states that hairs for medico-legal examination should be mounted dry in balsam, as preliminary treatment causes them to lose many characteristic features. From the many species examined (39) the following conclusions are reached: it is easy to distinguish between hairs of animals and other bodies of similar appearances. It is easy with practice to distinguish between human hair and that of lower animals; with a consideration of all the peculiarities the original site of the hair may be determined. From the appearances of the roots, if several specimens are available, it is possible to determine whether the hairs have fallen out or been plucked out. By the use of a polariscope a gray hair may be recognized from a pale blond. With crossed nicols blond hair gives a golden yellow band on a black ground, while a grey hair gives a parti-colored band, the red-purple predominating. In arsenic poisoning the metal passes into the hair; this was shown by injecting arsenic into animals hypodermically and testing the hair by the usual chemical methods. Control animals gave negative results.

A. M. C.

**Noll, A.** New Ether Freezing Apparatus for the Microtome. *Zeitschr. f. wiss. Mikr.*, 18 : 141-144, 2 figs., 1901. (*Fr. Journ. Royal Micr. Society*, 110-111, Feb., 1902.

Dr. A. Noll has devised a freezing apparatus, by which the necessary coldness is obtained by the evaporation of ether in a vacuum. It consists (Fig. 1) of a metal chamber K with two side pipes *a* and *b*, and a bar *c* for fixing to the microtome. The side pipes are connected by tubes *s*<sub>1</sub> *s*<sub>2</sub>; *s*<sub>1</sub> joins on to a funnel and is supplied with a stop-cock T; *s*<sub>2</sub> connects with a suction apparatus *p* (water pump) which exhausts the air in the chamber.

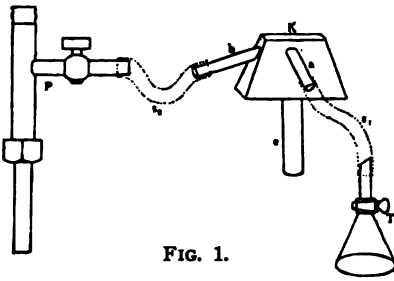


FIG. 1.

ments of the freezing box are: lower surface, 4–2.5 cm.; upper surface, 2.5–2 cm.; height, 3 cm.

A. M. C.

Wesenberg, G. Dropper for Sterile Fluids. *Centralbl. Bakt.* 30: 703–704, 1 fig., 1901. (From *Journ. Royal Micros. Society*, Feb. p. 116, 1902.)

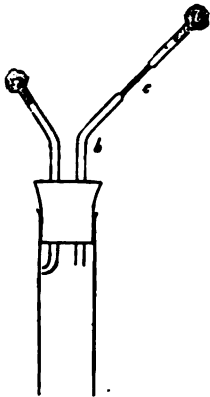


FIG. 2.

G. Wesenberg describes a dropping apparatus (Fig. 2) which is of simple construction and of especial advantage in disinfection experiments for washing the disinfectant out of the object to be tested. It consists of a caoutchouc plug, which is inserted into a test-tube or the neck of a flask. The plug has two perforations, one for a bent tube *a* through which air enters, and the other for the bent tube *b*. The latter is narrowed at *c* so that it can be broken off or sealed up in the flame. The way of using the apparatus is obvious. If the fluid does not flow freely it may be forced on by blowing through *a*.

There is little chance of air-infection if the end of *b* be held close to a flame when the tube is set upright after using it.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

Bradford, J. R., and Plimmer, H. G. The Trypanosoma Brucii, the Organism found in Nagana, or the Tse-tse Fly Disease. *Quart. Journ. Micr. Sci.* 45: 449–473, pls. 24–25, 1902.

The authors have studied this parasite in the blood and the various organs of the rat, mouse, rabbit, guinea pig, dog,

and cat. The flagellated adult stage should be studied under a cover-glass supported by a thin paraffin ring to obviate pressure, and sealed with paraffin to stop evaporation. The rapid movements of the organism may be checked by mixing a drop of 1 per cent. gelatin solution or a weak solution of cherry gum with the blood. The structure of the living organism, including the nucleus, micronucleus, vacuole, and alveolar protoplasm, is demonstrated by the use of dark ground illumination or monochromatic light. The former may be obtained even with a 2 mm. immersion objective, after the method of Gebhardt. Spectral monochro-

matic (blue) light was used, and very good results were obtained with Gifford's malachite green screen.

Ordinary methods of fixing and staining are of no avail in the preparation of *Trypanosoma*. Blood films must be made as thin and even as possible. This was accomplished by placing a drop of the affected blood on a corner of a cover-glass and spreading it with a piece of goldbeater's skin with straight edge and a width a little less than the cover-glass. Fixation by the vapor from a mixture of equal parts of glacial acetic acid and 2 per cent. osmic acid gave the finest results. Good results for general work were obtained with Gulland's mixture (absolute alcohol 10 parts, formalin 90 parts) allowed to act five or ten minutes, after which the specimen should be washed in running water and dried before staining. The only stain which proved to be of value is a modification of Romanowsky's mixture, prepared as follows: A 1 per cent. solution of methylen blue med. pur. (Höchst) is made in distilled water and .5 per cent. of potassium carbonate is added. The mixture is then incubated at 37° for forty-eight hours and filtered when cold. Instead of eosin the more uniform and constant erythrosin was employed. A .001 per cent. solution is made, and .25 per cent. formalin added to prevent the growth of moulds. The solutions are mixed for use as follows: 20 cm<sup>3</sup> of distilled water are placed in each of two beakers. To one of these 6 or 8 drops of the methylen blue solution are added and to the other 20 drops of the erythrosin solution. The two are quickly mixed and poured in a flat dish in which the preparations have been placed for staining. This is accomplished in 20 minutes. The preparations are then washed in distilled water till no more color comes away, and dried in the air without heat, and mounted in turpentine colophonium.

The organism appears in the blood of the rat and mouse forty-eight hours after inoculation. The spleen becomes greatly enlarged and contains plasmodial material, some amoeboid forms, and a few adult flagellated stages. The blood from the lungs contains many amoeboid stages, but few plasmodia or adults. The glands contain many amoeboid forms and a fair quantity of adults. Only the liver, kidneys, and bone-marrow contain adults in quantity corresponding with those in the blood. At death the cerebral capillaries are blocked with amoeboid stages. Different hosts exhibit different degrees of resistance to the parasite and its occurrence and distribution vary greatly, as does also the relative development of the different stages. Infection is fatal, killing rats in five days, while guinea pigs live for eighteen weeks. Phagocytosis of amoeboid stages was frequently observed. Removal of the spleen decreases this activity and hastens the fatal termination.

C. A. K.

Gough, L. H. The Development of *Admetus pumilio*, Koch: a Contribution to the Embryology of the Pedipalps. Quart. Journ. Mic. Sci. 45: 595-631, pls. 32, 33, 1902.

The embryos of this scorpion-spider were secured at Para. Methods of fixation and preservation are not given. Difficulties in sectioning caused by the yolk were overcome by the colloidin-paraffin

method as follows: The eggs were graded gradually to absolute alcohol and placed in celloidin for a number of days, then passed into chloroform and from this to paraffin and were then treated as ordinary paraffin objects. The sections were arranged on the slide by the water method, the water removed by blotting paper and then celloidin poured on the sections. This prevents shrinkage of the egg envelope or crumbling of the yolk in subsequent treatment of the slides in staining and mounting. The paraffin was removed by chloroform. Sections were stained in hæmatoxylin-eosin.

C. A. K.

Catels, E. H. Recherches sur l'Histologie et l'Anatomie Microscopique de l'Encéphale chez les Poissons. Bull. Sci. de la France et de la Belg. 36: 1-166, pls. 1-X., 1901.

As fixing agents the author used 95 per cent. alcohol, 10 per cent. formol, Flemming's chrom-osmic-acetic, Mayer's picro-nitric and Perenyi's chromo-nitric,

and especially recommends the last as excellent for nervous tissues. The stains used were Delafield's and Ehrlich's hæmatoxylin and various anilins, thionin being most highly recommended. Dogiel's method of using methylen blue was discarded after several unsatisfactory trials in favor of the following procedure: A concentrated solution of methylen blue (Ehrlich of Grübler) in normal salt solution was injected in the branchial vessels or in the dorso-lateral muscle masses. One or two cubic centimeters of this solution produces immediate intoxication, and at the end of a half-hour one finds the nerve centers more or less impregnated with the stain. The brain is then carefully removed and cut in four or five pieces in the sagittal or frontal plane. It is then placed for another half-hour in a saturated solution of the methylen blue and subsequently treated by the molybdate of ammonia method of Bethe or by the formol or platonic chloride method. The methylen blue method thus employed gave good results for the fibrillar zones of the optic lobes, the nerve fascicles and commissural fibers and the nuclei and radical fibers of the cranial nerves.

The Golgi method was used with good results, especially upon young animals. It is relatively easy to impregnate the following regions: the anterior and inferior lobes and at times the optic lobes and the superficial zones of the cerebellum. It is more difficult in the case of the olfactory bulb, the thalamencephalon, geniculate body, round nucleus, habenula, cerebellar peduncles, and deeper layers of cerebellum and cord. When the brain exceeded four millimeters in length it was cut in sections before immersing in the fixing fluid. After numerous trials the amount of osmic acid in the first bath was reduced below the customary proportion, the following solution being used:

3 per cent. bichromate of potash,	-	-	6 parts
1 per cent. osmic acid,	-	-	1 part

The duration of the first bath was 24-48 hours, and of the second (.75 per cent. silver nitrate) 36-48 hours. The Cox-Golgi method was also used with profit.

C. A. K.

Tower, W. L. Observations on the Structure of the Exuvial Glands and the Formation of the Exuvial Fluid in Insects. Zool. Anz. 25: 466-472, 8 figs., 1902.

The larva of the Chrysomelid beetle, *Leptinotarsa decem-lineata*, was employed in this study. The method used will be useful in other cases where cuticular

structures interfere with rapid penetration of fixing fluids and prompt fixation of tissues. Living larvae were placed for a minute or less in:

Saturated corrosive sublimate in 35 per cent. alcohol,	70 cm. <sup>3</sup>
Glacial acetic acid,	1 cm. <sup>3</sup>

This was heated to temperature of 60°-65°C. and the larvæ were quickly removed, cut in several pieces and placed in a weaker mixture:

Saturated corrosive sublimate in 35 per cent. alcohol,	99 cm. <sup>3</sup>
Glacial acetic acid	1 cm. <sup>3</sup>

The larvæ were allowed to lie in the fluid for thirty minutes to six hours, according to their size. A mixture of cedar-oil and xylol was used for clearing. Heidenhain's iron hæmatoxylin and Mayer's hæmalum followed by orange G or Zwaardemaker's safranin were used as stains. Material killed in Hermann's, Flemming's and von Rath's mixtures give good results, but less uniformly than the acetic-sublimate. Perenyi and the various picric acid mixtures were entirely unreliable and hot water only gave fair results.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

Verkes, R. M. A Contribution to the Physiology of the Nervous System of the Medusa *Gonionemus Murbachii*. Part I. The Sensory Reactions of *Gonionemus*. Amer. Jour. Physiol. 6: 434-449, 1902.

— A Contribution to the Physiology of the Nervous System of the Medusa *Gonionema Murbachii*. Part II. The Physiology of the Central Nervous System. Ibid, 7: 181-198, 1902.

In the first of these papers the author describes quite fully the reactions of *Gonionema* to chemical, mechanical and photic stimuli. While the tentacles are the most sensitive portions of the organism to all stimuli, yet all parts of the body with the exception of the exumbrella and the velum are sensitive

to both mechanical and chemical stimuli. The reactions to stimuli fall into two general classes, called by the author (1) the "feeding reaction" and (2) the "motor reactions," including as the most important subdivision the "locomotor reaction." The "feeding reaction" in its entirety is given only in response to chemical stimuli from substances of a nutritive nature. The reaction consists of a series of coördinated movements of the tentacles, bell and manubrium, which together bring about the carrying of the food substance to the mouth. To harmful substances the "locomotor reaction" is given. This reaction takes the organism quickly away from the harmful stimulus. The ability of the organism to react differentially to different chemical substances (foods and non-foods) indicates the possession of some power of discrimination or choice. The organism is found to be positively phototactic in daylight, and negatively photopathic to daylight and to greater intensities of light.

The purpose of the second of the papers is to determine the relation of the nervous system of *Gonionema* to its reactions. The author finds that the reactions of special parts or organs of the animal are not dependent on the activity of the central nervous system. Spontaneity is not dependent on the central nervous system, but upon the high degree of irritability of certain parts of the margin of the bell. The marginless bell does not show spontaneity simply because it is insensitive to any stimuli except those of considerable intensity. Yerkes develops a theory of coördination which makes the process independent of the functioning of the nerve ring or any special nerve center. According to the view advanced coördination is dependent simply upon the rapid transmission of an impulse, probably muscular rather than nervous in nature. All the tissues except the jelly and exumbrella are capable of transmitting impulses.

Together the papers form an important contribution to our knowledge of the behavior of a lower organism on which very little thorough work has previously been done from this point of view. It is to be noted that in the second paper the author, for etymological reasons, changes the generic name *Gonionemus* to *Gonionema*.

R. P.

**Cannon, W. B.** The Movements of the Intestine studied by Means of the Röntgen Rays. *Amer. Jour. Physiol.* 6: 251-277, 1902.

In 1898 Cannon published an account of the normal movements of the stomach of the living animal, studied by means of the Röntgen rays. The present paper is a continuation of that work and is devoted to an account of the normal movements of the intestines and their contents. The observations were made almost exclusively on cats, and the method of procedure was as follows: Subnitrate of bismuth, one-tenth to one-third the weight of the food, was mixed with what the animal ate. Canned salmon was used as food and the animals were not allowed to eat anything during the day preceding an experiment. After any desired interval following the feeding the animal was placed in a holder and exposed to the action of the rays. The outline and movements of any part of the alimentary tract containing the bismuth subnitrate could then be seen very clearly on the fluorescent screen. Records were taken, both by means of radiographs and by means of tracings made with a soft pencil on tissue paper laid over the fluorescent surface of the screen.

The author's more important conclusions regarding the movements of the intestine are as follows: The activity most commonly seen in the small intestine is the simultaneous division of the food in a coil into small segments, and a rhythmic repetition of the segmentation each time applied to the new segments formed from parts of those just divided. This segmentation may proceed at the rate of thirty divisions per minute. The segmenting activity serves primarily to thoroughly mix the food with the digestive juices, and to bring the digested food into close contact with the absorbing mechanisms. Peristalsis is usually combined with segmentation. The ileocecal valve is found to prevent perfectly the passage of the normal contents of the colon back into the small intestine, although allowing such passage in the case of material, whether fluid or mushy, introduced in large amount by the rectum. The usual movement of the transverse and ascending colon and the cœcum is an antiperistalsis. This recurs in periods about every fifteen minutes, and each period lasts about five minutes. The entrance of new food into the large intestine produces a strong general contraction along the cœcum and ascending colon, forcing some of the food onward; this contraction is immediately succeeded by the antiperistaltic waves. Deep tonic constrictions carry the material from the transverse into the descending colon. No evidence of antiperistalsis in the small intestine has been found. Signs of emotion, such as fear, distress or rage, were found to be accompanied by a total cessation of intestinal movements. The movements continue in the cat both during sleep and at night.

The paper certainly forms a noteworthy "contribution to a clear understanding of the normal movements of the intestine and their contents." R. P.

**Levene, P. A.** Recent Researches on the Chemistry of the Proteid Molecule. *Arch. of Neurol. and Psychopathol.* 3: 1-14, 1901.

A brief review and critical discussion of the recent literature on the chemistry of proteids. The author reaches the conclusion that but little progress has been made in this subject in recent years. R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Michaelis, L.** Ueber einem der Gruppe der Leukämie-artigen Erkrankungen zugehörigen Fall. *Zeitschrift für klin. Med.* 45: 87-96, 1902.

cells with a small amount of protoplasm. These cells are present in considerable number and are generally looked upon as lymphocytes. Michaelis, however, regards them as undifferentiated lymphoid cells. They cannot be distinguished morphologically from lymphocytes. The undifferentiated lymphoid cells by further development become transformed into neutrophilic myelocytes, while the lymphocytes are adult cells.

The undifferentiated lymphoid cell differs from Benda's "myelogenic" cell in that the former develops not only in the bone-marrow but in other tissues.

Michaelis supports his view by a remarkable case of disease of the blood-forming organs which came under his observation. The total number of leucocytes in the peripheral circulation was only slightly increased, but 76 per cent. of the cells were lymphocytes and 7 per cent. myelocytes. The patient died after an illness of three months. The lymph-nodes were not swollen. The spleen was very large and soft and weighed 2000 grams. The bone-marrow was red and consisted almost entirely of lymphocytes and giant-cells. The spleen had the same histological structure.

J. H. P.

**Robinson, G. C.** On a Cyst Originating from the Ductus Thyreoglossus. *J. H. Hosp. Bull.* 8: 81-82, 1902.

Cysts in the regio-thyroidea have been discussed by Verneuil, who collected a number of cases from the literature.

He found several bursae in the thyro-hyoid region, and thought cysts might come from them. The origin of one cyst, previously described, he attributed to the cystic dilatation of the pyramidal prolongation of the thyroid towards the hyoid.

In the development of the thyroid the ductus thyreoglossus, which is lined by ciliated epithelium, is cut off in its upper portion to form the blind lingual duct, while its lower portion forms the ductus thyroideus. Cysts have been described in both these portions, and even in the portion of the duct enclosed in the hyoid bone. Robinson relates a case. The patient had a rounded swelling in the median line of the neck, just above the thyroid region. Fluctuation could be detected on palpation. At autopsy the tumor was found to be a spherical, unilocular cyst about  $4\frac{1}{2}$  cm. in diameter. It lay upon and was lightly attached to the sterno-hyoidei muscles. By a narrow aberrant band of muscle it was connected with the lower surface of the body of the hyoid bone. On section its contents included a glairy mucoid fluid with a mass of cellular detritus. The lining surface was smooth and velvety. Microscopically the wall was composed of compact laminated connective tissue, with generally a lining cell, of a single layer of high columnar epithelium. No thyroid tissue was found, but from its position and lack of connection with either the trachea or pharynx, the given origin of the cyst seems most likely.

W. R. S.

**Sabrazes et Muratet.** Sarcomatose de L'Hy-poderme avec Généralisation Mésoderm-ique. *Archiv. de Med. Exper.* 14: 203-220, 1902.

Many multiple tumors of the skin have been described. The sarcomata form the largest class of these. They have

been separated by Perrin into (1) sarcomata, primary and secondary, melanotic in form, and (2) sarcomata non-melanotic. He further subdivides these two great divisions. In the second class we find the cutaneous and subcutaneous forms. The first variety has been well described by Kaposi and others. The second form presents marked differences. Its course is more rapid, and the prognosis is always fatal. Pautzier, Lapeyre and Labbè have given this class some attention, and Sabrazès and Muratet describe a case. The patient was a man, aged sixty-six, who presented many of these subcutaneous sarcomata. The onset was insidious, a small nodule being noted in the right subclavicular fossa. Other nodules were soon observed, especially over the abdomen and back. Death ensued in eight months. These tumors were accompanied by progressive emaciation, malaise, slight jaundice and marked anæmia. A vigorous treatment with arsenic improved this anæmia somewhat. At the autopsy nodules varying in size were found in the regions above noted, in the cellular adipose tissue of the kidneys, in the omentum, the pericardium, the myocardium and the cavities of the heart. They were only noted in mesodermic tissues. Microscopically these nodules were mostly made up of round and fusiform cells, though intermediate forms were also noted. The nucleus had generally one nucleolus, but several were at times seen. Occasionally two nuclei were in the same cells. Karyokinetic figures were quite abundant. Plasma cells were but rarely noted. The nodules were quite vascular, and extravasations of blood into the tissues were frequently observed. Hæmosiderin, the iron containing blood pigment, was present intra- and extra-cellularly in these nodules.

W. R. S.

**Zinno.** Ein seltener Blutbefund (Myelocytämie) in zwei Fällen von Pest. *Centralbl. f. allg. Path. u. path. Anat.* 13: 410-412, 1902.

Myelocytes in acute infectious diseases have been found by different investigators. Türk observed those with neutrophile granulations in many such

diseases, especially in pneumonia. Those with eosinophilic granules he found but rarely. French observers have worked in this field, but they do not give the variety of myelocytes found. Zinno relates two cases of bubonic plague which showed considerable numbers of eosinophilic myelocytes. In the first case, which resulted fatally, the blood was obtained on the third and fourth days of the disease. The red blood corpuscles were reduced to 3,800,000 while the leucocytes were 6000 (4000 of these being polymorphonuclears and 2000 mononuclears). The small mononuclear leucocytes composed about two-thirds of all the mononuclear forms, and eosinophilic myelocytes were present to the amount of 1-1.5 per cent. of all the leucocytes counted. In the second case the blood examinations were made on the eighth and ninth days of the disease. The red blood corpuscles were 3,600,000, while the white blood corpuscles were 38,000 (21,000 of these being polymorphonuclears and 17,000 mononuclears). Of the mononuclear forms the small mononuclears predominated. Eosinophilic myelocytes were present to the amount of 13 per cent. At times the eosinophilic leucocytes appeared to take their origin from them. The patient made a good recovery, and in a subsequent blood examination these leucocytes were found to amount to 13 per cent., while the myelocytes were very few. The presence of these eosinophilic myelocytes may be of some diagnostic importance.

W. R. S.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn, Wesleyan University, Middletown, Conn.

Beljerinck. Ueber oligonitrophile Microben.  
Cent. f. Bac. u. Par. II. 7: 561, 1901.

The author has given a valuable contribution to the problem of nitrogen assimilation. He has coined the word *oligonitrophile* to refer to micro-organisms which are capable of living in a medium containing very little combined nitrogen, and are themselves able to assimilate free atmospheric nitrogen. One or two species of such micro-organisms have previously been described, but the author discovers and describes certain new species with new properties. The most unique feature of his contribution is the fact that both chlorophyll holding plants and colorless plants are capable of assimilating nitrogen. Among the first stand certain species of the Cyanophyceæ group. These he studies by inoculating garden earth into flasks containing solutions prepared from chemicals, but containing no nitrogen, and carefully studying the growth of the micro-organisms. The chief plants which have this power belong to the species *Anabæna* and *Nostoc*. To find that these organisms are capable of assimilating free atmospheric nitrogen, is a new contribution to knowledge. The author, however, does not give any chemical tests to prove that there is actually an increase in the amount of combined nitrogen in the solutions. The rest of the paper is devoted to a study of the colorless organisms, of which he finds two new species of bacteria capable of assimilating free nitrogen and belonging to a new genus. This new genus the author names *Asotobacter*. It differs from those previously described in not producing spores. Two species of the genus are found, one living in garden earth, and the other is found in canal water. Both of them are motile organisms in their young condition, and neither produces spores. In each case the organisms are capable of living in solutions containing practically no nitrogen, and grow best under these conditions, assimilating atmospheric nitrogen. As a source of energy they are capable of using a large variety of carbon compounds. It is a curious fact, however, that when cultivated in pure cultures, they may grow luxuriantly in the presence of a considerable quantity of combined nitrogen, differing thus from some of the other nitrogen assimilating bacteria.

To this same general group of organisms, which he calls oligonitrophiles, the author classifies the bacilli which produce the tubercles in the roots of legumes.

H. W. C.

Sullivan, M. X. Some Experiments with  
Synthesized Media. Abstract of paper read  
at 3d Ann. Meet. of Soc. of Am. Bact.

Pasteur, Cohn and others recognized that some bacteria can secure their carbon, hydrogen, oxygen and nitrogen from simple compounds. This is to be expected from the close relationship of the bacteria to other plants. Recently the question of simple synthesized media has received some attention from Muntz, Jordan, and de Schweinitz. Since Pasteur, however, little use has been made of media other than those made of meat-infusion and peptone with agar or gelatin as a base. Meat-infusion and commercial peptone vary so widely in their chemical composition and in their nutrient value, that media composed of these substances are practically of no use in the study of pigment of antitoxin production, or in the study of bacterial metabolism.

Analysis of standard bouillon shows such small amounts of albumoses and peptones that it appears as if we might neglect the meat-infusion entirely, or at least replace the peptone by a non-nitrogenous, non-albumenous body.

On medium (A), consisting of water 100 grams, Witte's peptone 5 grams,

NaCl 3 grams, agar 1 gram, seventeen different kinds of bacteria, including *Microspira comma*, *B. anthracis*, *B. typhosus*, and *B. coli*, grew more slowly than on the standard media. Chromogenic bacteria, as *B. pyocyaneus* and *M. pyogenes citreus*, however, failed to produce pigment. On medium (B), made of water 100 grams, peptone 6 grams, NaCl 2 grams,  $MgSO_4$  3 grams,  $K HPO_4$  0.5 grams, agar 2 grams, fifteen varieties grew quickly with the production of pigment and, in one case, of phosphorescence.

Then the peptone was replaced by a non-albumenous compound such as ammonium salts or asparagin. On medium (C), containing water 100 grams, glycerin 50 grams,  $(NH)_4 PO_4$  10 grams,  $Na HPO_4$  1 gram,  $MgSO_4$  0.2 grams, agar 1 gram, nineteen varieties grew rather slowly. On medium (D), consisting of water 100 grams, asparagin 1 gram, NaCl 0.5 gram,  $MgSO_4$  0.3 gram, agar 1 gram, nine varieties grew with no formation of pigment. On medium (E), consisting of water 100 grams, asparagin 1 gram,  $Na PO_4$  0.1 gram, NaCl 0.2 gram, agar 1 gram, twenty different kinds of bacteria, mostly pathogenic, grew as quickly as on the standard media.

Further experiments are now being carried on, and, in view of the fact that so many bacteria can grow on this non-albumenous medium, it is probable that some combination of simple chemicals can be found that will replace the ordinary meat-infusion.

A medium consisting of such compounds, qualitatively and quantitatively known, would be of great value in the study of bacterial metabolism. H. W. C.

Arloing, M. The Innoculability of Human Tuberculosis and Robert Koch's Ideas concerning Human and Animal Tuberculosis. Bulletin of the Academy of Medicine, session of Dec. 24, 1902.

After referring to the opinion that Robert Koch expressed at the Congress of London, and the principles laid down by this distinguished German bacteriologist, and upon which he based his

assertion of the difference between human and bovine tuberculosis, the author gives to the academy an account of his experiments, and the conclusions he has drawn from them. Koch has established numerous differences between human tuberculosis and that of cattle; these differences are admitted by all bacteriologists; but he falsely makes the assertion that human tuberculosis is entirely different from that of ruminating animals. According to Koch, 1st, animals of the bovine, ovine, and porcine species cannot be innoculated with the human bacillus, while the bacillus of bovine tuberculosis easily infects these animals; 2d, the bovine bacilli do not produce tuberculosis in human beings.

We print herewith a short resumé of the numerous experiments by which Arloing refutes these statements. The author has succeeded in infecting bovine, ovine, and porcine animals with the bacilli of human tuberculosis. The bacilli used were grown from human lesions, and had passed once or twice through the guinea pig. The innoculation was made into a vein, for Koch had declared that the results were identical whatever the method employed. Thus, Arloing has succeeded in infecting a calf, a bull, a heifer, a sheep, and a horse. Of these animals some succumbed, others stood the infection moderately well; but in all cases there were found lesions, pulmonary, ganglionic, hepatic, splenic, renal, etc., in fact a general condition of tuberculosis.

The negative facts found by Koch and other authors depend upon the method of innoculation, the dose, and the virulence of the tubercular culture used. Arloing has shown, in fact, that the tubercular bacillus presents considerable variations in its virulence, and that this same virulence may be modified by cultivating the virus under certain conditions.

The identity of tuberculosis ought to be maintained, and it is still necessary to take precautions with regard to the milk and flesh of tubercular animals. The experiments of Arloing lead to the same conclusions as do those of Nocard.

Translated by Eleanor L. Lattimore.

A. GIRAULD.

## NEWS AND NOTES.

**MOUNTING OBJECTS FOR TEMPORARY STUDY.**—Objects which are to be examined only temporarily may be mounted in water or, if it is preserved material, in a drop of the preservative. Alcohol and formalin, however, evaporate too rapidly to serve for more than very short observation. Normal salt solution (.6 gram common salt in 100 c. c. water) serves well for a great many objects. Whenever living objects, such as micro-organisms, are to be examined, they should be mounted in the natural medium, as a change of medium may retard the activities of the organism. If too much liquid is placed on the slide, the cover, when dropped into place, will float about, or the specimen may float out from under the cover. Surplus liquid should be removed with a cloth or blotting paper until the cover remains in position, even when the slide is inclined. On the other hand, if too small an amount of liquid is placed on the slide or too much withdrawn, the specimen, if it be a soft object, may be crushed. The cover should not be allowed to fall upon the drop of medium, for one is then unable to control the position of the specimen and air bubbles are apt to be formed. The cover should be held with fine forceps and one edge brought in contact with the liquid, then gradually lowered into position, with the object as near the center of the field as possible. To prevent evaporation of the medium, if one wishes to keep the specimen for some time, the cover may be sealed to the slide with a ring of vaseline or castor oil.

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**ON THE TEACHING OF PLANT PHYSIOLOGY TO LARGE ELEMENTARY CLASSES.\***—Until each student can be supplied with apparatus for the various experiments in plant physiology, the presentation of the subject to large classes will be more or less difficult and unsatisfactory. It is generally agreed that, in elementary work, eight to twelve conclusive and practicable experiments upon the most fundamental subjects, as photosynthesis, respiration, osmotic absorption, etc., are sufficient. Professor Ganong's plan for meeting the difficulties involved and attaining the end desired, is as follows: "I try first to make sure that the importance and general bearing of the problem to be studied is clearly before them; in fact, I try to make the experiment seem, as it really is, not only a logical but a necessary step in their progress. Then I set up the experiment from the very beginning, explaining the reason for each step and for the use of each piece of apparatus, the action of each chemical, and the physical principles involved. The students follow, asking what questions they wish, making full notes and sketches, etc. The completed arrangement is then placed in the laboratory, and each student makes individual records of the observable results. When the results are complete, or when the result depends upon some special test to be applied, the experiment is again brought before the class; the tests are applied and results discussed. A mimeographed synopsis of the experiment, including the theory of the method, and giving such statements of fact as are essential to the full grasp of the subject, is then supplied to them, and from these various sources they are expected to prepare a synoptical, illustrated exposition of the object, method, results and bearings of the experiment.

C. W. J.

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\* Ganong, W. F., Smith's College. School Science, 1: 9.

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## The Bacterial Flora of Freshly Drawn Milk.

### I.

The constant presence of bacteria in freshly drawn milk is a matter of considerable importance, and this fact goes a long way in explaining the ineffectual attempts to obtain milk in commercial quantities uncontaminated by bacteria. At the same time, it has been but very recently that investigations as to the number and nature of the organisms that gain access to the milk through their localization and multiplication in the milk ducts have been made. The first recorded experiments are those of Leopold Schultz<sup>1</sup> in 1892. He examined milk bacteriologically at the first milking, in the middle of the milking, and at its close. This examination consisted merely in counting the number of bacteria present; and, as a result, the following figures were determined: The first milk contained from 55,000 to 97,200 germs per c. c., the middle milk from 2000 to 9000 germs per c. c., and the last milk was in some cases sterile, and sometimes contained about 500 germs per c. c. The number of germs in the last milk he says depended upon the quickness with which the milking was done. When done quickly, all the germs were washed out, so that "the last milk was often but not always sterile."

Gernhardt,<sup>2</sup> investigating the same subject, found a larger number in samples from the middle of the milking than at the beginning. To explain this result as well as to explain irregularities in the numbers, he suggests that the bacteria made their way up through the milk ducts of the teats, through the cistern and into the smaller ramifications of the ducts which connect the cistern with the ultimate follicles. As many of the colonies so formed are not easily removed, they are not found in the first milk, but appear later when they have become broken up by the movements of milking.

Von Freudenreich,<sup>3</sup> on the other hand, states that when in the udder, milk is free from bacteria except when the milk glands are in a diseased condition.

H. L. Bolley and C. M. Hall,<sup>4</sup> in their studies of the bacterial flora of the

milk of ten healthy cows, isolated 16 distinct species of bacteria, some of which were common to both the first and last milk, and others to only one of these. All the micro-organisms found were bacteria, and none were found which produced gas.

H. L. Russell,<sup>5</sup> in his text-book on Dairy Bacteriology published in 1894, states that he has found an average of 2800 germs per c. c. in the fore milk, while the average of the remainder of the milk only had 330 germs per c. c. In characterizing these, he says that "the number of species is usually small, one or two kinds usually predominating to a large degree. Those that are commonly found are those that produce lactic acid, as these microbes find in milk the best medium for their growth."

Gosta Grotenfeldt,<sup>6</sup> however, in his text-book on the Principles of Modern Dairy Practice, reasserts the statement of von Freudenreich, that when the milk is drawn from the udder of a healthy cow, it is germ-free, or sterile.

Dr. T. M. Rotch<sup>7</sup> concludes, from the examination of the bacteria found in four cows' milk, that the bacteria do not necessarily come from external sources, but that they may also come from some part of the milk tract between the udder and the end of the teat. The few colonies, however, obtained in the plates from the latter half of the milkings are considered as possible contaminations between the "cow" and the "plates."

V. A. Moore<sup>8</sup> states that in investigations made upon this subject, he found that, in addition to the bacteria in the fore milk, the last milk from at least one-quarter of the udder in every case contained bacteria.

H. W. Conn,<sup>9</sup> reviewing this subject, says that the different results of many of these early experiments is due to the small quantities of milk taken, while in the latter experiments large quantities have been taken. He adds, "Undoubtedly the milk gland of the healthy cow produces milk which is uncontaminated with bacteria, but the large calibre of the milk ducts makes it possible for bacteria to grow in the duct to considerable extent, so that it becomes a matter of extreme difficulty to obtain milk from the cow, even with the greatest precautions, which shall not be contaminated."

Harrison,<sup>10</sup> in a report of investigations upon this subject, states, "When milking is done, there remains in the teat of the cow a little milk that affords nourishment to any bacteria that may come in contact with it through the opening at the end of the teat." The average of a number of analyses made by him shows the presence of 18,000 to 54,000 germs in the fore milk, and 1000 to 3000 in the after milk.

Experiments have also been conducted on human milk by Palleske,<sup>11</sup> Honigmann,<sup>12</sup> Knochenstiern,<sup>13</sup> and Ringel,<sup>14</sup> and all of these have independently found it impossible to get human milk from the mammary gland in such a way as to be sterile.

The most recent work upon this subject has been done by Veranus A. Moore and Archibald R. Ward<sup>15</sup> of Cornell University. They investigated the source of a gas and taint-producing bacterium in cheese curd, in 1897, for a certain factory that was troubled with "gassy curd." They easily located the trouble in the herd of a particular patron. On inquiring into the history of the herd, it

was ascertained that at the time of parturition the placenta had been retained by a number of cows, and these had been allowed to decompose in the uterus. It was soon after this that the "gassy" curd began to appear. A thorough bacteriological examination located the bacillus which was the cause of the "gassy" curd in the udders of the cows of the herd; and it seemed very probable, though of course not demonstrable, that it had gained access to the udders from the decaying placenta.

Subsequent to this, Ward conducted further experiments, and in an article on "The Persistence of Bacteria in the Milk Ducts of the Cow's Udder,"<sup>16</sup> he concludes (1) "certain species of bacteria are normally persistent in particular quarters of the udder for considerable periods of time, and (2) it is possible for bacteria to remain in the normal udder and not be ejected along with the milk." These conclusions contravert the statement previously made by Von Freudenreich and Grotenfeldt that the milk ducts are always sterile at the close of milking, becoming tenanted from the outside alone by organisms which chance to come in contact with the end of the duct.

The results of still later investigations by the same author are published in a bulletin<sup>17</sup> on "The Invasion of the Udder by Bacteria." In these investigations, a bacteriological examination was made of the udders of milch cows slaughtered after reacting to the tuberculin test. In all cases the udders were perfectly normal. Just before slaughtering the animals were milked as thoroughly as possible, and samples of the milk taken and a bacteriological examination made. After slaughtering, a similar examination was made of the tissues of the udder. In all cases, even in the upper third of the udder, bacteria were found, and they were identical with those found in the milk. He concludes that "milk, when secreted by the gland of the healthy udder, is sterile. It may, however, immediately become contaminated by the bacteria which are normally present in the smaller ducts of the udder." However, "the bacteria so far found in the interior of the udder apparently do not affect milk seriously. This, however, does not preclude the possibility that forms more injurious to milk may invade the udder."

From the above resumé it is apparent that widely different results have been obtained by various investigators, and it has been a very interesting study to see whether the experiments conducted at Guelph would throw any light upon these divergencies in results.

The plan of experiment has been as follows: For a number of days samples were taken from the fore and after milk of a number of cows on the college farm. The samples were collected in sterile test tubes, and previous to taking the milk, the flank, udder, and teats of the cows were thoroughly washed with a 1-1000 solution of mercuric chloride. Gelatin plates were then made from these samples, and afterwards the number of colonies counted and the different species isolated and cultivated on the various media. It soon became apparent that while several species were more or less constant in the udders of all the cows, yet there were many variable species present in the milk of some cows that were not present in that of others, and not even in the same udder on two successive days. Therefore, in making a systematic study, it was deemed best

to confine our attention to those species that were more or less constantly present in the milk of all the cows, and to make a complete study of those existing in the udder of one particular cow.

The numbers of bacteria present in both the fore and after milk of the various cows, and of the same cow, and even in the different quarters of the udder of the same cow were so widely different that little stress can be laid upon an exact enumeration.

The following examples, which are typical of numerous others, will illustrate the point:

Cow No. 1.			Determination 1.	
Fore milk, right front teat,	-	-	86,400	per c. c.
" " " hind "	-	-	120,000	"
Strippings, " front "	-	-	40,800	"
" " " hind "	-	-	57,600	"
			Determination 2.	
Fore milk, right front teat,	-	-	48,000	per c. c.
" " " hind "	-	-	24,080	"
" " left front "	-	-	22,400	"
" " " hind "	-	-	35,100	"
Cow No. 2.			Determination 1.	
Fore milk,	-	-	200-500	per c. c.
After milk,	-	-	0-100	"

The results of a large number of determinations, of which the above are typical, showed on an average 25-50,000 germs per c. c. in the fore milk. The numbers in the strippings, or after milk, varied greatly with the manner of taking. For example, when the milking was done quickly, but very few and sometimes no colonies were found in the "strippings;" whereas, when the milking was done slowly and some time lost before the samples from the last milk were taken, the number of bacteria was very variable, being in one case as high as 57,000.

The important point, therefore, is not the exact number, but the fact that bacteria were found in large numbers, not only in the fore, but in the middle and last milk of nearly all the cows tested.

The number of species present in the udders of cows is comparatively small. Of this number, some are more or less constantly present, whereas others are very variable in their presence. Of those species which are present, the characters are in many cases so slightly marked that their identification proved a very difficult matter. In fact, with the exception of *Bacillus acidi lactici*, not a single species discovered was strongly characterized. A number had a very slow or no effect upon milk, and even the digestors were in every case very slow digestors. But this matter will be more fully discussed after a description of the species has been given.

*B. acidi lactici* (Conn, No. 206), *B. acidi lactici* (Conn, 202), and *B. lactis aerobans* (Conn, 197), Nos. I, II, and III, are the only ones that have been found constantly present in all the samples, and in every case they have composed at least 95 per cent. of the germs present. The species to be subsequently described have been only more or less variably present, and in no case have been found in large numbers.

IV. *B. halofaciens* (n. sp.).

*Occurrence*: Of frequent occurrence in fore and after milk.

*General Characters*: Shape and arrangement—Bacterium occurring singly.

Size—Short, about  $1\frac{1}{2} \times 1$ .

Motility—Motile.

Spore formation—None.

Relation to temperature—Grows at room temp., but optimum temp. is  $37^{\circ}$ .

Relation to air—Aerobic and facultative anærobic.

Relation to gelatin—Slow liquefier.

Color—White, dirty brown, and finally yellow.

Stain—Stains evenly with anilin dyes.

*Gelatin*: Stick culture—Beaded growth to bottom of tube, with an abundant surface growth, at first white but afterwards becoming yellow. At first there is a slight crateriform liquefaction with yellowish deposit and a white halo around edges. After 12 days liquefaction becomes complete.

Plate culture—Surface col.; colony is at first white, but soon becomes brownish, and in about three days intensely lemon-yellow, and the colony, which is about 5 mm. in diameter, becomes surrounded with a whitish halo extending out into the gelatin.

*Agar*: Streak culture—A thin, waxy, transparent yellow growth.

*Milk*: A weak alkaline curd is formed in four days, with yellowish ring on top. Subsequent digestion is slow but complete in about three weeks at  $37^{\circ}$  C.

*Potato*: A moderately thick lemon-yellow growth, somewhat spreading.

*Smith Tube*: No gas produced in either glucose or lactose.

*Synthetic Media*: No reduction of nitrates.

*Bouillon*: Becomes cloudy in one day at  $37^{\circ}$ , and a heavy yellowish deposit collects.

This bacterium approaches in characteristics *Bact. annulatum*, Wright (19), but differs from it in several details, so that we do not hesitate to call it a new species. The name refers to the characteristic halo found in gelatin cultures. As this bacterium was of quite frequent occurrence, we made butter from cream ripened with a culture of it, and found that the flavor of the butter, while not strong, was quite disagreeable. At the same time, its presence in relatively small numbers as compared with Nos. I and II, and the fact that the flavor was not by any means strongly marked, makes this an inconsiderable item so far as the "natural ripening" of such milk is concerned.

V. *Micrococcus varians lactis*. Conn, 113 and 104. (21.)

*Occurrence*: Quite frequent in both fore and after milk.

*General Characters*: Shape and arrangement—Micrococcus occurring singly.

Size—Rather large;  $1 \mu$  in diameter.

Motility—None.

Spore formation—None.

Relation to temperature—Grows readily at  $18^{\circ}$ , but best at  $37^{\circ}$ .

Relation to air—Aerobic and facultative anærobic.

Relation to gelatin—Slow liquefier.

Color—Varies from white to orange.

Stain—Stains very easily with ordinary aniline dyes.

*Gelatin*: Stick culture—Slight plumose growth along needle track. Liquefaction infundibuliform, at first dry with a yellowish deposit. Gradually liquefaction spreads, becoming horizontal, and a cloudy liquid is formed with heavy orange precipitate.

Plate culture—Surface col.; at first a whitish bead, smooth at center, becoming yellowish with age. Liquefaction slight or none. Colony about 5 mm. in diameter.

*Agar*: Streak culture—A white to yellowish, glistening, smooth, moist, abundant growth along line of needle.

*Milk*: Curdles in three days at 37° into a soft curd with amphoteric reaction. No subsequent digestion, but a clear, watery fluid settles out on top. Occasionally a slight digestion.

*Potato*: A dry, granular, raised growth, not spreading. Color varies from pure white to orange.

*Smith Tube*: No gas produced in either glucose or lactose.

*Synthetic Media*: Reduction of nitrates marked.

*Bouillon*: In two days becomes cloudy. After four weeks, a yellow sediment is deposited, but the liquid is still cloudy.

Conn, in speaking of this micrococcus, considers it one of the most important of dairy species, and suggests that it likely exists in the milk ducts.

#### VI. B. No. 18, Conn (?).

*Occurrence*: Almost constantly present in all samples.

*General Characters*: Shape and arrangement—Bacillus occurring singly and in twos.

Size—Short and thick, about one and one-half times long as broad.

Motility—None.

Spore formation—Terminal spores found in old gelatin and agar cultures.

Relation to temperature—Grows at room temp., but optimum is 37°.

Relation to air—Aerobic and facultative anærobic.

Relation to gelatin—Non-liquefier.

Color—White.

Stain—Stains evenly without ordinary aniline dyes.

*Gelatin*: Stick culture—An abundant plumose growth along needle track, with a thick, white, cream-colored surface growth.

Plate culture—Surface col.; an opaque white bead about 1 mm. in diameter, smooth and round.

*Agar*: Streak culture—An abundant thick, white, moist growth.

*Milk*: No effect is produced on milk in three weeks. After four weeks it becomes slightly acid, and a slow coagulation takes place.

*Potato*: Slight light brown, or cream-colored growth, raised, but not spreading.

*Smith Tube*: No gas produced in either glucose or lactose.

*Synthetic Media*: No reduction of nitrates.

*Bouillon*: In one day, at 37°, a slight turbidity, and afterwards a heavy white deposit is formed.

This species appeared very frequently in all samples examined, but never in very large numbers. In old gelatin and agar cultures, spores appeared at the ends of the bacilli. Two cultures from these heated for 10–15 minutes at a temperature of 85°–90° germinated in one day. The species is very similar to *Bacillus* No. 18, Conn, but differs in that growth on potato is not spreading, and no spores are found in potato cultures.

#### VII. *Bacillus* No. VII.

*Occurrence*: Of variable occurrence in milk examined.

*General Characters*: Shape and arrangement—*Bacillus* occurring singly and in chains.

Size—Variable. From 3 to  $1\frac{1}{4}$  times as long as broad.

Motility—Motile, but not actively so.

Spore formation—Observed in old gelatin cultures.

Relation to temperature—Grows at room temp., but optimum is 37°.

Relation to air—Aerobic and slightly facultative anærobic.

Relation to gelatin—Slow liquefier.

Color—White to yellowish.

Stain—Stains evenly with aniline dyes.

*Gelatin*: Stick culture—Faint beaded growth along line of puncture. Liquefaction crateriform, a thick, tough, white scum being formed on top. After a few days the liquefaction becomes horizontal with a scum on top and a deposit on the bottom.

Plate culture—Surface col.; large, flat, round, raised colonies, with a dense white to yellowish nucleus, and a clearer outer granular zone marked by concentric circles.

*Agar*: Streak culture—An opaque porcelain colored, waxy growth, not spreading much.

*Milk*: A hard, homogenous, solid, acid curd, with no gas, is formed in two days at 37°. In some cases there is no separation or digestion. In other cases there is very slight digestion.

*Potato*: Extensive growth, dry, elevated, and somewhat reticulated, and creamy to yellow in color.

*Smith Tube*: No gas produced in either glucose or lactose.

*Synthetic Media*: No reduction of nitrates.

*Bouillon*: In one day at 37° becomes uniformly turbid, after which a white scum is formed on top and a deposit below.

This bacillus was quite constantly present for some weeks, but afterwards disappeared. It seems to resemble *B. cremoris* (18) (*B. lactis* No. 9, Flügge), but differs in its effect on milk, so that it would appear to be an allied species.

#### VIII. *Bacterium* No. VIII.

*Occurrence*: Of variable occurrence in milk examined.

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*General Characters*: Shape and arrangement—Bacterium occurring singly.

Size—Very short, but thick.

Motility—Actively motile.

Spore formation—None.

Relation to temperature—Grows at room temp., but optimum is 37°.

Relation to air—Aerobic and slightly facultative anærobic.

Relation to gelatin—Slow liquefier.

- Color—White.

Stain—Stains evenly with aniline dyes.

*Gelatin*: Stick culture—Slight beaded growth along needle track. Liquefaction at first crateriform, and then horizontal, a white scum being formed on top, and a white deposit on the bottom.

Plate culture—Surface col.; large, round, flat, white, regular colony, with a dense, white, nuclear center, and a clearer outer zone.

Under the microscope the nucleus is granular and the outer zone reticulated, except the narrow-boundary margin, which is structureless.

*Agar*: Streak culture—An opaque, porcelain colored, moist, glistening, slightly spreading growth.

*Milk*: Milk rendered alkaline and slightly curdled in three days at 37°, and then slowly digested.

*Potato*: At first a moist spreading growth, scarcely discernible. Afterwards it becomes thicker, brownish, but remains moist. It is not reticulated.

*Smith Tube*: No gas produced in either lactose or glucose.

*Synthetic Media*: Slight reduction of nitrates.

*Bouillon*: In one day at 37° becomes turbid. In four weeks there is a white, irregular scum on top and a white deposit. After six weeks the liquid becomes clear.

The gelatine plate colony appears very similar to No. VII, but the organisms otherwise differ, both morphologically and culturally, in many particulars. Like No. VII, it appeared quite constantly for some weeks, but since then has disappeared.

#### IX. *B. exiguum*. Wright (19).

*Occurrence*: Found quite constantly in the milk of one cow.

*General Characters*: Shape and arrangement—Bacterium occurring singly.

Size—Medium size, about two and one-half times as long as broad.

Motility—None.

Spore formation—None.

Relation to temperature—Slow growth at room temp., optimum growth at 37°.

Relation to air—Aerobic and facultative anærobic.

Relation to gelatin—Slow liquefier.

Color—Ferruginous to light red.

Stain—Stains evenly with ordinary aniline dyes.

*Gelatin*: Stick culture—Slight arborescent growth along line of needle, but

not reaching to the bottom. Liquefaction infundibuliform and very slow and dry, with a reddish deposit on sides. After 6 to 8 weeks the liquefaction becomes horizontal, more liquid accumulates, and a red deposit is formed.

**Plate culture**—Surface col.; growth slow. After two days a small, round, white colony, which after 4 to 5 days develops into a colony about 2 mm. in diameter, round and smooth. Under the microscope it appears granular with a large opaque center surrounded by a more transparent ring, while the outside ring is transparent.

**Agar**: Streak culture—Abundant, moist, smooth, opaque salmon-colored growth in 24 hours at 37°. With age, it becomes slightly ferruginous in color.

**Milk**: After three days, at 37°, there is a reddish-colored ring at the top. In five days a soft curd is formed of amphoteric reaction. Subsequent digestion is slow, and not complete for 4 or 5 weeks. Litmus milk decolorized.

**Potato**: Abundant spreading, reddish to ferruginous-colored, moist growth.

**Smith Tube**: No gas produced in either glucose or lactose.

**Synthetic Media**: No reduction of nitrates.

**Bouillon**: Growth slow at all temperatures. After three days the liquid becomes turbid, and a white sediment is deposited.

This bacterium was found almost constantly present in the milk of one of the cows tested, but was never found in that of any other. There were never more than one to four colonies per plate present, so that its effect on the milk was very inconsiderable. Its similarity to *Bact. exiguum* of Wright (19) is most marked. Wright isolated this bacterium from water; but the fact that he found its optimum temperature to be 36° and that it is a facultative anærobo, does not make it at all surprising that it should be found in the udder of a cow.

#### X. *Micrococcus* No. X.

**Occurrence**: Found occasionally in the milk examined.

**General Characters**: Shape and arrangement—*Micrococcus* clustering in twos and fours.

Size—of medium size.

Motility—None.

Spore formation—None.

Relation to temperature—Grows at room temp., but optimum is 37°.

Relation to air—Aerobic and facultative anærobic.

Relation to gelatin—Slow liquefaction.

Color—White.

Stain—Stains evenly with ordinary aniline dyes.

**Gelatin**: Stick culture—Slight beaded growth about half way to the bottom. Liquefaction very slight, crateriform; thick, white, beaded deposit on surface.

**Plate culture**—Surface col.; a granular colony with more liquid outer zone. Soon the granular center becomes broken, and the

whole center of about 5 mm. becomes filled with granular masses which frequently project beyond the rim.

*Agar*: Streak culture—An abundant, raised, porcelain white, moist growth, not spreading.

*Milk*: Slight effect. A slight white deposit is formed in litmus milk, and after a long period of 4 or 5 weeks the milk becomes thinnish. Reaction alkaline.

*Potato*: A beautiful opaque, porcelain white, dry chain of bead-like, much raised colonies.

*Smith Tube*: No gas produced in either glucose or lactose.

*Synthetic Media*: No reduction of nitrates.

*Bouillon*: In one day, at 37°, becomes cloudy. In six weeks it is still cloudy, and there is a slight white deposit.

This coccus comes in the same class as Conn's 167, and may be identical with it. The most marked variations are the gelatin colony, and the fact that in no culture of this germ was there the slightest indication of a yellow color. At the same time it agrees in morphological characters, and especially in the fact that, although a liquefying coccus, it fails to curdle milk.

Like several of the forms previously described, this coccus was found to be present for some time in the samples taken, but afterwards in a few weeks completely disappeared. In no cases was it present in large numbers.

Ontario Agr. College.

F. C. HARRISON,  
M. CUMMING.

### •An Improved Method for Making Collodion Tubes for Dialyzing.

The use of a collodion tube for dialyzing is so convenient, so cheap, and so accurate as to commend itself for more general use, both to the chemist and to the biologist.

The following method of preparing these I have found much the easiest, and by it tubes of any desired size may be made very rapidly. Into test tubes, or tubes of that pattern of the desired size, pour enough three per cent. collodion so that by inclining the tube and twirling it the sides will be completely coated. Pour back the superfluous collodion, still twirling the tube a few seconds to allow the collodion film to begin drying. Turn the tube mouth down over a coarse screen, or prop it up on two blocks, so that the excess collodion may drain easily and also to allow free access for the air to dry and harden the film. Allow to stand from five minutes to one hour, then fill the tubes with water, and in a few minutes the collodion tube is loosened and may be easily drawn out from the test tube and is ready for use. The longer drying makes a tougher tube, the shorter drying a quicker one. A much thicker solution of collodion may be used if stronger and slower tubes are desired. These tubes will keep for some time in water. If exposed long to the air, they become shrunken and rather brittle.

KARL KELLERMAN.

Bureau of Plant Industry.

## A Review of the Methods of Staining Blood.

### II.

#### B. *Fixing Dry Blood Films.*

The fixing processes applied to dry blood films are for the purpose of coagulating the albuminous substance of the blood which cements the corpuscles to the cover-glass and prevents them from being washed off or the hemaglobin from being dissolved out of the red corpuscles in the process of staining. The treatment for this purpose varies with the method of staining. Many of the eosin-methylen blue neutral compounds require no previous fixation, this being accomplished in the process of staining. The neutral tri-color stain of Ehrlich and its modifications require but slight previous fixation. On the other hand, the basic dyes and especially the acid dyes require thorough fixation before staining. Moreover, different stains require different methods of fixation. The basic dyes act more satisfactorily after chemical fixatives, but the acid dyes should be preceded by dry heat, and many staining solutions require special methods of fixation. Fixation of dry blood films is accomplished by the various application of two agents, dry heat and chemicals.

#### DRY HEAT.

Dry heat is the fixative used by Ehrlich for dry blood films. Various ways have been devised for applying the proper amount of heat.

1. **Passing the Preparation through the Flame.**—This is the method of bacteriologists for fixing dry preparations of bacteria to the cover-glass. The cover-glass, held by forceps, is passed three or four times slowly through the flame of a Bunsen burner or alcohol lamp. It rarely gives satisfactory results for blood preparations.

2. **Heating above the Flame.**—This method gives better results than the preceding, but is not to be recommended except in case of haste. The cover-glass is held by its edges between the thumb and forefinger as low over the flame of the Bunsen burner as one can bear the heat for thirty seconds.

3. **Heating on a Metal Plate.**—The flame of a Bunsen burner or an alcohol lamp is placed under one end of a metal plate supported on a tripod stand. After an equilibrium between heating and radiation has been attained, the preparations are placed blood side downward at a position on the plate a little nearer the flame than where a drop of water is at once converted to steam, and allowed to remain from fifteen minutes to an hour.

*Rubinstein* (1897-8) endeavored to determine a position of optimum temperature for quickly fixing blood films on the metal plate. If a drop of water be allowed to fall on the metal plate sufficiently near the point of application of the heat it will be converted into steam; at points nearer to the flame the quicker it will be vaporized until a position is reached where the drop instead of being vaporized rolls about on the plate. This point Rubinstein calls the "spheroidal zone" and the point of optimum temperature for fixing dry blood films. The preparations are to be placed blood side down on this zone so that an edge of

the cover-glass is somewhat outside of the zone in the direction of the cold end of the plate. One-half to three-fourths of a minute suffices to fix the preparations.

4. **Heating in Thermostat.**—A dry oven with thermo-regulator and thermometer attachment gives the most uniform and satisfactory results in dry heat fixation. The temperature is variously given from 110° to 135° C. It is probable that preparations will not be spoiled within those limits, and a mean of the two limits suggests itself until more definite experiments have determined the optimum temperature. The time of heating is given from fifteen minutes to three hours, depending upon the temperature and the method of staining to be used.

*DeCosta* (1902) describes an oven for the rapid fixation of dry blood films. He raises the temperature to 160° C. The gas is then turned off and the cover of the oven thrown back, and after the temperature has fallen to 30° C. the films are ready to stain.

#### CHEMICAL FIXATIVES.

1. **Alcohol.**—Hlava (1883), Malachowsky (1891), Deetjen (1901), and others have used 96 per cent. of absolute alcohol for fixing dry blood films. Fixation is complete in a few minutes. Goldhorn (1901) fixes fifteen seconds in pure methylic alcohol.

2. **Alcohol and Ether.**—Nikiforoff (1888), and subsequently many others, recommend absolute alcohol and ether, equal volumes, as a fixative. Fixation is complete in fifteen to thirty minutes.

3. **Alcohol followed by Formalin.**—Deetjen (1901), for demonstrating the nuclei of blood plates, fixed dry preparations in 98 per cent. alcohol 1 to 2 minutes, dried in the air, then in 5 per cent. formalin solution 3 to 5 minutes (this solution should be old and have stood in the light several weeks) and washed in water without drying.

4. **Chloroform.**—Josné (1901) recommends chloroform for fixing blood preparations.

5. **Chromic Acid.**—Harris (1883) and Müller (1889-91) used chromic acid as a fixative. Müller used  $\frac{1}{2}$  per cent. chromic acid for ten days, and then washed in running water for twelve to twenty-four hours.

6. **Flemming's Solution.**—Löwit (1883) and Müller (1889-91) used Flemming's solution. Both first fixed with dry heat and then with Flemming's solution one to six hours and washed well with running water.

7. **Formalin.**—Benario ( ), Edington (1900), and Japhà (1901) employed formalin. *Benario* recommends a 1 per cent. alcoholic solution in which the dry preparations are fixed one minute. *Edington* describes a simple apparatus for fixing dry blood films with formaldehyde. A bell-jar 135 mm. in diameter and 150 mm. in height, having a stopper at the top, to the inner end of which is glued an ordinary cover-glass, is placed over the air-dry blood preparations resting on a glass plate. The stopper of the jar is removed, a drop of formalin is placed on the cover-glass glued to its end, and the stopper quickly replaced in the top of the jar. The cover preparation, which must be thoroughly dry, remains from fifteen to thirty minutes in the formalin vapor. *Japhà* fixed air-dried preparations with 1 to 2 per cent. formalin-alcohol for one minute.

8. **Hermann's Fluid.**—Müller (1892) used Hermann's fluid for the study of the nuclei of leucocytes and nuclear structure.

1 per cent. platinic chloride,	-	-	-	15
2 " " osmic acid,	-	-	-	4
Acetic acid,	-	-	-	1

Fix either fresh or dry preparations several days to a month (Müller recommended ten days), wash in running water twelve to twenty-four hours, stain.

9. **Mercuric Chloride.**—Hlava (1883), Kruse (1890), and Van der Strecht ( ) fixed dry preparations in sublimate solution. *Kruse* used a concentrated watery solution, in which the preparations remained five to ten minutes, then five minutes in absolute alcohol to which was added iodine tincture, five minutes in pure alcohol, stain. *Van der Strecht* fixed the air-dried preparations twelve to twenty-four hours in a 2 per cent. solution of sublimate in a physiological salt solution, then one hour in absolute alcohol with iodine, several hours in pure absolute alcohol, washed in water, stained.

10. **Mercuric Chloride-Alcohol.**—Argutinsky (1901) used a sublimate-alcohol solution for fixing blood films for the study of malarial parasites. Seven grains of pure sublimate are dissolved in 100 c. c. of a 1 per cent. hot aqueous solution of sodium chloride, and when cool 100 c. c. of absolute alcohol is added. The dry preparations are subjected to this fixative for five to eight minutes, then washed in strong alcohol for a few minutes and transferred to iodine alcohol. This solution is prepared by adding 1 gram of pure iodine to 100 c. c. of absolute alcohol. After rinsing in absolute alcohol and drying between filter paper, the preparations are ready to stain.

11. **Picric Acid.**—Müller (1891), Hock and Schlesinger (1892), and Mannaberg (1893) used picric acid fixation. Müller, in his study of leukæmic blood, fixed heated dry preparations twenty-four hours in a concentrated watery solution of picric acid, washed in running water 12 to 24 hours, and stained.

12. **Picric Acid and Acetic Acid.**—Mannaberg (1891) and Hock and Schlesinger (1892) employed a picric and acetic acid mixture. Mannaberg gives the following formula :

Saturated watery solution of picric acid,	-	-	30
Distilled water,	-	-	30
Glacial acetic acid,	-	-	1

Fix dry preparations for two hours in this solution.

13. **Zenker's Fluid.**—Whitney (1901) recommends a modified Zenker's fluid for fixing dry blood films. Zenker's fluid, in which 5 per cent. of strong nitric acid is substituted for the 5 per cent. of glacial acetic acid, is allowed to act on the film for a few seconds (count 20), wash with running water. Ehrlich's "triacid" solution, Una's polychrome methylen blue and Chenzinski's eosin and methylen blue mixture are said to work well after this fixation, but Löffler's alkaline methylen blue gives a fine precipitate on the red corpuscles.

### III. STAINING THE PREPARATIONS.

The stains used in staining the blood may be divided into three groups depending upon the position of the active staining property in the dye. Dyes in which a staining base is combined with an inert acid as methylen blue, methyl

green, thionin, etc., are called basic stains; those in which a staining acid is combined with an inert base, as eosin, orange G., aurantia, acid fuchsin, etc., are called acid stains. To these two general classes of stains Ehrlich has added a third, which are especially important in the staining of blood. Stains of this class are produced by adding a solution of a basic dye in proper proportion to a solution of an acid dye. A precipitate results which is believed to consist of the staining base of the basic dye united with the staining acid of the acid dye. This compound dye is called by Ehrlich a neutral stain.

Of the elements of the blood, the nuclei, the granules of certain of the leucocytes (mast cells), the cytoplasm of the lymphocytes, the cytoplasm of the immature red corpuscles before hemoglobin development, and malarial parasites and bacteria when present, have a varying affinity for the first class of stains, the basic stains, and, following the generally accepted terminology, may be called *basophile*. The discoplasm of mature red corpuscles, the granules of certain leucocytes (acidophiles) and the cytoplasm of the leucocytes have a varying affinity for the second class of stains, the acid stains, and may be called *acidophile*. The neutral stains, being a compound of basic and acid dyes, stain both basophile and acidophile elements and in addition stain the granules of a third variety of leucocytes in human blood which are not stained by either the basic or the acid stains, and which are called *neutrophile*.

#### A. Basic Stains.

##### 1. Dahlia.

*Ehrlich's* staining fluid for the granules of the mast cells consists of:

Absolute alcohol,	-	-	-	-	50 c. c.
Distilled water,	-	-	-	-	100 c. c.
Glacial acetic acid,	-	-	-	-	12.5 c. c.
Dahlia to thorough saturation.					

The preparations should be floated on the surface of this staining solution for twenty-four hours, and then washed and examined either in water or dried and mounted in balsam. All of the basophile elements of the blood are stained, including the granules of the mast cells which are not stained by many of the basic dyes.

*Goldhorn* (1902) recommends wood alcohol saturated with dahlia for staining mast cells. The staining solution is poured on the fresh blood smear without previous fixation. The preparation is then washed with water and dried in the air.

##### 2. Dahlia and Carmin.

*Westphal* (1880), who worked under Ehrlich, used the following dahlia mixture: 100 g. c. Carmin of Porfsch-Grenacher (pure carmin 2.0, distilled water 200.0, alum 5.0; cooked one-fourth hour, filtered and 1.0 carbolic acid added), 100 c. c. of glycerin, 100 c. c. of strong alcoholic solution of dahlia, 20 c. c. of acetic acid.

##### 3. Haematoxylin.

*Mannaberg* recommends the following alum hæmatoxylin solution for staining malarial blood: A fairly old solution of 10 grains of cryst. hæmatoxylin

in 100 grams of absolute alcohol. Just before use 1 part of this solution is mixed with 2 parts of a one-half per cent. solution of ammonia alum.

*Rees* (1901) recommends Delafield's hæmatoxylin or Mayer's hæmalum for staining malarial blood.

#### 4. Methylen Blue.

This is the most important basic stain that we have for staining the blood. It is a precise basic stain, staining only the basophile elements, and does not overstain. It forms the basic part of the modern eosin-methylen blue stain for the blood.

*A Concentrated Watery Solution* of methylen blue stains all of the basophile elements of the blood except the granules of the mast cells.

*Löffler's Alkaline Methylen Blue* solution is a better blood stain than the watery solution. It is especially recommended for staining bacteria, malarial parasites and the basophile cytoplasm or granules of red blood corpuscles. The formula is as follows :

Concentrated alcoholic solution of methylen blue, - 30 c. c.

Caustic potash in 1:10,000 solution, - - - - 100 c. c.

Preparations which are best fixed in equal volumes of absolute alcohol and ether should be stained from one to five minutes or more. It does not overstain. The stained preparations are washed off with water and may either be examined directly in water or dried and mounted in balsam.

*Sahl's Borax Methylen Blue* solution is recommended by Malachowsky (1891) for staining malarial parasites of the blood. Dry preparations are fixed in absolute alcohol for several minutes and then stained for twenty-four hours in :

Concentrated watery solution of methylen blue, - 24

5 per cent. solution of borax, - - - - 16

Water, - - - - - 40

Filter after twenty-four hours.

*Rees* (1901) gives another formula :

Pure medicinal methylen blue, - - - - 2 per cent.

Borax, - - - - - 5 per cent.

Distilled water, - - - - - 93 per cent.

Mix. Filter before use. Stain thirty to fifty seconds. The stain improves by keeping.

#### 5. Methyl Green.

*Arnold and Howell* (1891) recommend methyl green in 0.6 per cent. sodium chloride for staining the nuclei and nuclear division in fresh blood. The blood cells are fixed as well as stained by this solution.

#### 6. Methyl Green and Fuchsin.

*Ehrlich* recommends the following double basic stain: A saturated watery solution of methyl green is mixed with an alcohol solution of fuchsin. This stain requires only slight fixation and only a few minutes' action. Nuclei are stained green, red corpuscles red (diffuse), protoplasm of lymphocytes fuchsin color. It is especially recommended for demonstrating lymphatic leukæmia.

### 7. Methyl Violet and Rubidin.

*Griesbach* (1890) stained dry blood films shortly with methyl violet, rubidin; washed, dried, mounted.

### 8. Safrinin.

*Leigh* (1888) allowed air-dried preparations to swim face downward in a half saturated solution of safrinin in absolute alcohol.

### 9. Thionin.

*Marchoux* (1897) gives the following formula for a thionin stain for malarial blood:

Concentrated alcoholic solution of thionin (60 per cent. alcohol),	20 c. c.
2 per cent. watery solution of carbolic acid,	- - - 100 c. c.

This mixture is used after standing fifteen days, then it stains in a few seconds. The leucocytes and parasites are stained red-violet.

*Rees* (1901) gives this formula:

Thionin blue,	- - - - -	1.5 grams
Absolute alcohol,	- - - - -	10 c. c.
Aqueous solution of carbolic acid, 5 per cent.		100 c. c.

Keep for two weeks, or longer. Filter before use, and dilute with distilled water 1 in 4. Staining takes place in about 10 minutes. Protoplasm of malarial parasites stains as intense purple, nuclei of leucocytes blue, red corpuscles a faint greenish blue.

*Craig* (1901) gives still another formula after *Fletcher*:

Saturated solution of thionin in 5 per cent. alcohol,	20
Two per cent. carbolic acid,	- - - - - 100

The solution should stand several days before using. Red corpuscles stain a slight greenish hue, parasites a deep violet.

### 10. Toluidin.

*Rees* (1901) gives the following formula for toluidin blue stain for malarial blood:

Toluidin blue,	- - - - -	5 grains
Sodium carbonate,	- - - - -	4 "
Distilled water,	- - - - -	100 c. c.

Dissolve the sodium carbonate in water gently warmed, stir in the toluidin blue. Keep a few days, and filter before using. Stain 30 seconds.

## METHODS IN PLANT PHYSIOLOGY.

## V.

## IV. ROOT HAIRS.

(a) Plant seeds of white mustard (*Sinapis alba*) or of buckwheat (*Fagopyrum esculentum*) in a crock of sand, allowing water to stand constantly in the saucer beneath the crock. As soon as the seedlings appear above the surface loosen the sand with a knife-blade and withdraw the seedlings. Observe how the particles of sand are held by the root-hairs. (b) Germinate some pop-corn seeds in damp sawdust, when they have attained a length of 3 or 4 cm. set up three or four seedlings in a damp chamber and a like number in a jar with their roots immersed in water. After two or three days observe the seedlings for the presence of root-hairs, noting their absence from the roots in water.

## V. PHYSICAL OSMOSIS AND IMBIBITION.

1. **A Convenient Osmometer.** Slip a short piece of rubber tubing over the small end of a thistle-tube and close the rubber tubing with a clamp. Fill the thistle-tube with a 20 per cent. sugar solution and tie a piece of wet animal membrane or parchment paper over the bowl of the tube. Invert the thistle-tube, immersing the bowl in water, remove the piece of rubber tubing and mark the height of the sugar solution in the tube, which should be about 2 cm. above the bowl. If it is necessary to add more solution after the tube is inverted, use a fine pointed pipette, allowing the solution to run down the sides of the tube in a thin stream instead of drops. If air bubbles form in the tube they may be broken up by inserting a fine wire. Support the tube with a clamp at such a height that the liquid in the tube and the water outside are at the same level. Record the rise of the solution in the tube every five hours.

2. **Artificial Cell.** Smooth the ends of a short glass cylinder by grinding or by fusion; over one end tie firmly a piece of wet animal membrane or parchment paper, fill the cylinder with a 20 per cent. sugar solution, and while the cylinder is lying in a basin of sugar solution close the open end with another piece of membrane, excluding the air. Place the cylinder in a dish of water for 24 hours, then examine the membrane for a change in tenseness. If one of the membranes is pricked with a needle and the needle quickly withdrawn, the spurting of the liquid will indicate to some extent the amount of pressure.

3. **Traube's Cell.** The osmotic action through a precipitation membrane is well demonstrated by the familiar example of Traube's cell. Place a crystal of copper acetate or copper chlorid in a small vial containing a 5 per cent. solution of potassium ferrocyanide, immediately set the vial on the table and watch the growth of the membrane of cupric ferrocyanide, which is at first globular then cylindrical.

4. **The Rate of Diffusion.** Slip a short piece of rubber tubing over the small end of a thistle-tube, double the rubber tubing back upon itself and insert it into a large test-tube nearly filled with water. Pour a few cubic centimeters of potassium dichromate solution into the thistle-tube, by careful manipulation allow a

little of the potassium dichromate solution to flow out into the water at the bottom of the test-tube. Let the thistle-tube remain in the test-tube, mark the upper limit of the colored solution, and leave the preparation where it will be undisturbed for several days; each day mark the movement of the solution, calculate the rate of diffusion.

5. **Effect of Temperature upon Imbibition.** Weigh out two sets of beans or peas of 30g. each. Place one set in water maintained at 30°C., the other in water at 10°–15°C. for three to five hours. At the end of that time turn off the water and dry the seeds on a towel, weigh and determine the amount of water absorbed in each case.

6. **Effect of Dissolved Substance upon Imbibition.** (a) At the time of preparing the last experiment weigh out an equal quantity of seeds and place them in a 10 per cent. salt solution. Keep this experiment at the temperature of one of the two in the last experiment for the same length of time. Dry the seeds on a towel, weigh and compare the increase in weight in the salt solution with that obtained in the water.

(b) Instead of seeds, blocks of dry wood may be used for the same purpose. Make some cubes of oak wood 2 cm. on each edge, measure them accurately in the radial and tangential directions with millimeter calipers; place one cube in a 10 per cent. salt solution, one in distilled water, and one in 96 per cent. alcohol. At the end of twelve to twenty-four hours measure each block accurately and determine the proportionate increase in size due to imbibition.

7. **Rise of Temperature During Imbibition.** Place in a wide mouthed bottle 100–125 c. c. of starch which has recently been dried at a temperature of 30°–40°C. Stir the starch with the bulb of a thermometer until it reaches room temperature. Pour upon the starch an equal volume of water of the same temperature as the starch, stir quickly with the thermometer and note the rise in temperature within fifteen seconds after the addition of the water. If an excess of water is avoided a rise of 6°C. may be obtained.

## VI. PHYSIOLOGICAL OSMOSIS.

1. **Plasmolysis.** (a) Cut a small tangential section from the lower surface of a leaf of *Tradescantia discolor*, mount it upon a slide in a 3 per cent. aqueous solution of potassium nitrate. Observe immediately with a microscope the plasmolysis of the parietal layer of protoplasm, determining the limits of the vacuole by the aid of the colored cell-sap.

Observe a single cell in which the plasmolysis shows distinctly, draw off the potassium nitrate with filter paper, at the same time adding water, notice the increase in the size of the vacuole and the return of the protoplasm to its former position.

(b) Test gently with the finger the root of a seedling 3–6 cm. long for its rigidity. Lay the seedling in a 3 per cent. solution of potassium nitrate for ten minutes and test again, when it has become flaccid lay it in tap water for ten minutes and note the return to turgidity.

2. **The Osmotic Strength of Cell Sap in Terms of Potassium Nitrate.** Cut a column 3 or 4 cm. long from a petiole or flower stalk of the calla (*Richardia*) or

hyacinth (*Hyacinthus*). Divide this column into at least five longitudinal sections each of which shall have a strip of epidermis on one side. Lay each strip of tissue on a piece of paper and with a soft lead-pencil quickly draw a line to indicate the length and curvature of each strip, then place a strip in each of five potassium nitrate solutions, .5 per cent., 1 per cent., 1.5 per cent., 2 per cent., 2.5 per cent. After 10–20 minutes remove the strips from the solutions, lay each one beside the line corresponding to it and draw another line to indicate the change in curvature. The strip which has lain in the solution which is just isotonic will show no change in curvature, those in solutions weaker than the cell sap will show greater curves, those in solutions stronger than the cell sap will straighten as a result of plasmolysis.

8. **Tissue-tension.** Cut a leaf-stalk of *Richardia* 20 cm. or more in length and measure its length precisely. With a sharp scalpel cut a superficial longitudinal slice about 2 mm. in thickness the whole length of the stalk. After a few minutes it will be found to be several millimeters short of its original length. Next remove the entire epidermis from the central column of pith and determine its increase in length.

The transverse tangential tension may be illustrated by taking a transverse disk 3–4 mm. in thickness and separating, by means of a sharp scalpel, a ring of outer tissue 2 mm. in thickness from the inner tissue. While the outer ring still encloses the center, cut through the former and place them both in water. After 15 minutes the outer ring is no longer sufficient to enclose the center.

## VII. TRANSPIRATION.

1. **Lifting Power of Evaporation.** Tie a piece of animal membrane over the bowl of a thistle-tube while the tube is wholly immersed in water; without allowing any air to enter, support the tube in an upright position with the small end standing in a dish of mercury. The mercury will rise in the tube until its weight draws in air through the membrane.

2. **Lifting Power of Transpiration.** Fill a U-tube with water, support it in an upright position, and in one end fasten a stem of some plant, using a rubber stopper and allowing the cut end of the stem to project a centimeter beneath the stopper. The stem should be cut off under water and kept in water until placed in the final position in order to keep the cells cut across from filling with air. Into the open end of the U-tube pour mercury until it stands 3 or 4 cm. higher in the open arm of the tube than in the closed arm. The weight of the mercury column will inject the stem with water. Allow the preparation to stand until the stem wilts, noting the rise of mercury in the closed arm at regular intervals.

Fig. 5. A Potometer.

The horizontal glass tube is 60–75 cm. in length. (Not shown in the figure.)

3. **Rate of Transpiration Measured by the Potometer.** Construct a potometer as shown in Fig. 5; the outer end of the long glass tube should be several centimeters higher than where it bends to pass through the cork. The whole apparatus should stand on a tray which can be carried from one place to another. The rate of transpiration can be calculated by measuring the recession of the water in the long arm of the glass tube. Inject a leaf of the horse-chestnut (*Aesculus Hippocastenum*) or a plant stem, as in the preceding experiment, tie it firmly into the short piece of rubber tubing on the T-tube. When the experiment is working well make the following tests:

- (a) the rate of transpiration in a cool atmosphere, 15°–20°C.
- (b) the rate of transpiration in a warm atmosphere, 23°–28°C.
- (c) the effect of wind on transpiration; in this test the temperature of the wind must be taken into account.

4. **Rate of Transpiration Measured by Weighing.** A potted plant is to have the pot enclosed in rubber sheeting or oiled paper, the sheeting being tied around the base of the plant stem, thus preventing evaporation of moisture from crock and earth. The plant is then to be balanced on a weighing scale and a comparative study made of the rate of transpiration in cool air, in warm air, and in a current of air as in the preceding experiment. Finally determine the effect on transpiration of putting a bell-jar or a wrapping of paper over the plant.

5. **Restoration of the Transpiration Current.** Allow two horse-chestnut leaves or cut stems to become somewhat wilted, place one with its cut end in a bottle of water, fill a U-tube with water and firmly fasten the other leaf in one end of the tube. Inject the plant by pouring mercury into the open end of the U-tube until there is a mercury pressure of 5–7 cm. The injected plant will revive in less than an hour, the time should be noted and compared with that required for the uninjected plant.

6. **The Path of the Transpiration Stream.** Place a translucent stem or a stem bearing white flowers in a jar of dilute aqueous solution of eosin or methylene blue. If no stems of Impatiens or Begonia can be obtained use seedlings of Indian corn which have attained a length of 15–25 cm. After half an hour the colored liquid can be detected in the fibro-vascular bundles of the plant.

7. **Influence of the Epidermis on Transpiration.** Select two apples and two potatoes, paring one of each; weigh each object carefully, then set all away in the same place for twenty-four hours or more. At the end of this period weigh out again and compute the loss of weight.

8. **The Function of Stomata on Transpiration.** Determine by microscopical examination some plant whose leaves have stomata on one surface only. Soak a few square centimeters of filter paper in an aqueous solution of cobaltous chlorid and dry it in an oven or over a radiator; when the paper is dry note its color, then notice the change in color produced by moist vapor, e. g., steam or the breath.

From the paper cut two circles 1–2 cm. in diameter and fasten them by means of cover-glasses and weak wire clamps to the opposite sides of a leaf of the plant selected. Within a few minutes the paper covering the stomata will be bleached by the aqueous vapor escaping therefrom, while the paper on the opposite side will retain its color.

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## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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### X. EXAMINATION OF THE BLOOD.

It is only within the last few years that so-called "Blood work" has been done by the medical profession in general. The diagnostic value of blood examinations is well established and instruments for the purpose are now found in the office of every progressive medical man.

A general examination of the blood should include an estimation of the blood cells both red and white, estimation of the hæmaglobin, a general

Thoma-Zeiss Hæmacytometer, for counting red and white blood corpuscles.

microscopical examination and an examination for the plasmodium of malaria.

For the estimation of the blood cells, commonly called a blood count, a Thoma-Zeiss Hæmacytometer with pipettes for both red and white corpuscles and the following solutions will be required:

1 oz. 95 per cent. alcohol.

1 oz. Sulphuric ether.

1 oz. Toison's fluid, which is made as follows:

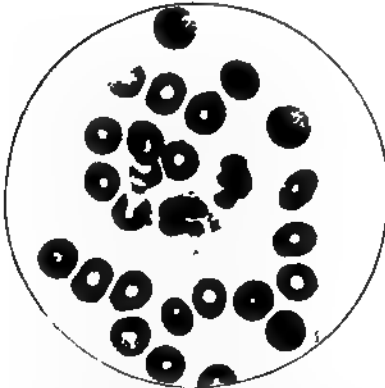
Sodium sulphate - - - 8 grams

Sodium chloride - - - 1 gram

Glycerine, pure - - - 30 grams

Aqua distilled - - - 160 c. c.

Methyl violet - - - .025 grams



Normal Human Blood. Stained with hæmatoxylin and eosin. Red cells and polynuclear leucocytes are shown magnified 1200 diameters.  $\frac{1}{3}$ -inch oil immersion objective, Bausch & Lomb compensating photo ocular No. 2.

Clean the finger or lobe of the ear with soap and water, alcohol, and lastly with ether. Dry thoroughly and puncture with a blood lancet. The drop of blood must exude without pressing the tissues. Wipe away the first blood, using the second drop for the count. Suck blood into the pipette marked 1 and 101, up to the mark 1. Wipe off the adherent blood on the point and draw the pipette full of Toison's solution to the mark 101, thus filling the bulb of the instrument completely. Shake well that the glass ball may thoroughly mix the blood and the salt solution. One-half of this mixture should be blown out of the instrument. A

small drop should now be placed on the small ruled space of the counting chamber. Apply the cover immediately to insure accurate results. If too large a drop is used it may get between the cover and the outer supporting cell. If the drop is too small the ruled space may not be filled properly. In either case, clean the chamber and cover and try again. Set aside to settle. The pipette should be cleaned at once to prevent coagulation of the blood in the capillary tube. Blow out the blood solution in it. Draw it part full of distilled water, blow this out, then use alcohol and finally ether in the same way.

For counting the prepared slide an objective of the same power as the Bausch & Lomb one-fifth should be used. Count the cells in the squares, beginning at the upper left corner. Count all of the cells on the upper line, the line to the left as well as those in the square. Cells on the lower line and the line to the right will be counted with the next line of squares. The cells in 100 squares should be counted to arrive at accurate results. To compute the number of red cells per cubic millimeter divide the number of cells by the number of squares counted, the result will be the average number of cells in one square. Multiply this by 4000 as each square is 1-4000 of a cb. mm. Multiply this result by 100 as the blood has been diluted 100 times. For example, suppose 100 squares are counted, the number of cells being 1260— $1260 \div 100 = 12.6$ , the number of cells per square.  $12.6 \times 4000 = 50,400 \times 100$ , the dilution = 5,040,000, the number of cells per cubic millimeter.

To count the white corpuscles proceed in the same way except that blood should be drawn into the pipette marked 1 and 11, to the 1 mark, followed by the diluent, which should be a one-half per cent. solution of acetic acid in distilled water, to the mark 11. Thoroughly mix and put a drop in the counting chamber and proceed as in counting red cells. The acid solution will destroy the red corpuscles and render the white corpuscles more distinct. The computation of the white corpuscles in one cb. mm. of blood is the same as for red except that last multiplication should be by 10, as the dilution is but 10 times.

The centrifuge method of estimating the quantity of plasma and red cells is sufficiently accurate for general purposes. Examinations may be made from fresh blood and from diluted blood which may be conveyed a distance, the examinations being made some hours later.



Centrifuge Percentage Tube. Twice actual size.

To examine fresh blood, clean a finger with soap and water, alcohol and ether. Dry thoroughly. Puncture with a blood lancet and fill one of the percentage tubes. A large drop of blood is required. The tube will fill automatically if the opposite end to the one in the blood is depressed. Insert the tube in the hæmatokrit and revolve immediately for three minutes at a speed of from eight to ten thousand revolutions per minute. Multiply the number of divisions filled with red corpuscles by 100,000. The result will be the number of red corpuscles per cubic millimeter of blood. When it is necessary to carry the blood from the patient to the office, proceed as follows to dilute and preserve the blood. Draw blood into a capillary tube to a certain height, and blow it into a small clean vial in which an equal amount of a 2.5 per cent. solution of potassium bichromate in water has been previously measured and placed. Mix thoroughly. Fill a percentage tube with this mixture and revolve the same as fresh blood. Multiply the number of spaces filled by 100,000 and then by two on account of the dilution.

WILLIAM H. KNAP.

## The Technique of Biological Projection and Anesthesia of Animals.

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### VIII. THE ANESTHESIA OF ANIMALS.

Before entering into the details of the methods of mounting various types of live animals and plants and the forms of cells best adapted to hold them on the projection microscope while they are being used in the production of living charts, it is desirable that the methods of producing anesthesia should be explained. The present tendency in biological teaching to emphasize the study of live organisms in their natural habitat or engaged in their normal activities in the more or less artificial environment of the laboratory, is worthy of the largest recognition and universal adoption, but the practical impossibility of controlling active animals on the laboratory table, or stage of the microscope, has been a serious obstacle to the attainment of a desirable degree of definiteness in these lines of work. By the use of a suitable anesthetic, nearly all the animals studied in elementary courses in zoölogy and a large number of other species may be very easily put to sleep and in this state are peculiarly adapted to careful study under the hand lens, compound microscope, and projection microscope. The degree of quiet may be varied from true hypnosis, or the condition of sleep, in which the voluntary muscles only are passive and all other parts are normally active, through different degrees of partial to complete anesthesia, and to the death of the animal, if this is desired. The hypnotic condition is most desirable in ordinary studies, since it is more quickly induced, is seldom, if ever, fatal, and while it lasts, if no pain is inflicted on the animal, the voluntary muscles remain passive and limp, affording the best conditions for the study of normal structures, functions, and such vital phenomena as the pulsation of the heart and the actual movement of its valves. By carefully grading the strength of the anesthetic, animals may be kept for extended periods of time on the border line between normal activity and sleep, and at the conclusion of the experiments revived and used again and again during the same or succeeding days. The methods are so simple and the anesthetising agent so safe for students to use that they readily learn how to do the work and accomplish satisfactory results. It may be remarked, in passing, that the lively interest awakened in the students as they use these methods is a pedagogic factor of no small importance.

It is not our purpose to enter into a discussion of the relative values of the different chemical compounds which have been used with varying success in the anesthesia of the lower animals, but to proceed, at once, to the consideration of the one believed to be the best and having the widest range of usefulness. A few years ago a new hypnotic, called Chloretone, was brought to the attention of the medical fraternity by its manufacturers, Parke, Davis & Co., of Detroit, Mich. In the spring of 1900 a description of the chemical and of its use on the human subject awakened the hope that it would give better results than other agents in the control of animal motion during projection experiments.

The first trials of Chloretone were so successful that a series of careful studies extending through several weeks were undertaken to determine the range of its usefulness and the formulæ for its use on a large number of species of animals from the Protozoa to Amphibians, and in a minor surgical operation on a valuable dog. The results exceeded all expectations as to its value in the projection of live animals, and also led to its introduction into regular laboratory work. The methods of use were demonstrated to the teachers of biology in the Chicago high schools in the fall of the same year, and were included in a course of instruction in Biological Projection in the University of Chicago in the summer of 1901. On the basis of the experience of the writer and the satisfactory results reported by other teachers who have used it, the Chloretone method of anesthesia is offered as a useful addition to laboratory methods in zoölogy, as well as an indispensable adjunct in the projection of live animals for the purpose of careful study or while being photographed.

Chlorotone, having the formula  $C_4H_7OCl_3$ , is a white crystalline compound with an odor and taste resembling camphor. It is usually obtained in bulk in the form of fine crystals for laboratory use, but is also sold in sugar-coated tablets and in capsules, which is its most convenient mode of administration to some of the lower vertebrates. The crystals are added to clear water, not distilled, at ordinary room temperature, and in the proportion of one part Chloretone to ninety-nine of water by weight or Chlorotone crystals one gram, water 99 c.c. A solution of approximately the same strength may be prepared by adding enough of the crystals to water to form a saturated solution. The crystals dissolve slowly and two days, or more, are required for the solution to reach its maximum strength of one per cent. The solution should always be made up and kept in a tightly stoppered bottle on account of the volatility of the Chloretone, and the crystals need the same care. The one per cent. stock solution remains unchanged for months. In use it is diluted with tap, well, or river water, to the degree of strength required by the animals which are to be treated. With a few species, the undiluted solution, the tablets, or capsules are used. At first thought the expense seems to be great, as Chloretone crystals cost about one dollar per ounce; but this amount will make about three quarts of one per cent. stock solution, four and a half gallons of the strength most commonly used, and the average cost per student per year will be less than five cents.

The usual mode of administering Chloretone to aquatic animals is by immersing them in a solution of suitable strength. The tray or other container in which the animals are placed should be chosen with a view to having them completely covered, when a relatively small amount of solution is added. Place the animals, one or enough for an entire class, in a tray, and pour on them a sufficient quantity of solution of the proper strength to cover them, or, if more convenient, place the animals in a measured quantity of water and add enough one per cent. stock solution to bring it to the right degree of dilution. The action of the Chloretone is such that great accuracy is not necessary in ordinary work. If the animals are distributed to the members of a class for examination and are found to be either too slightly or too profoundly anesthetised, one or more drops of stock solution may be added or the solution diluted with water,

as the case may require. The complete control of the organs of locomotion and of the mouth parts, thus easily obtained, will be appreciated by many who are engaged in research work. A valuable characteristic of Chloretone is that it does not, in most animals, produce contraction of the muscles, but they remain limp and expanded. It is extremely useful, therefore, alone or followed by a killing agent in the preparation of well expanded museum and laboratory specimens. The time required for inducing the hypnotic state or complete anesthesia varies widely in different species, depending, apparently, on the absorbing power of the general surface of the body and gills, if the latter are present, the observed minimum being fifteen seconds in large, active amoebæ and the maximum forty-five minutes in a medium-sized crayfish.

A. H. COLE.

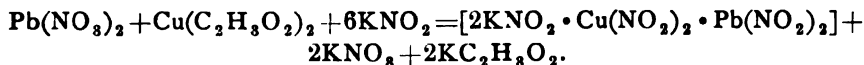
University of Chicago.

## MICRO-CHEMICAL ANALYSIS.

## XX.

## SILVER GROUP CONTINUED—LEAD.

*V. In the presence of a salt of Copper and of Potassium Nitrite, Lead forms a Triple Nitrite of Potassium, Copper and Lead of low solubility.*



*Method.* To the moderately concentrated neutral test drop add a trace of acetic acid, then a fragment or two of sodium acetate and of copper acetate. Stir. Then add a fragment of potassium nitrite. In a few seconds cubes and squares and rectangular plates separate which are brown by reflected light, jet black by transmitted light (Fig. 84). Very thin plates appear brown by transmitted light.

*Remarks.* This reaction is an extremely interesting and valuable one, though in the form described is not so delicate as some of the other tests for lead. If, however, we add to the test drop after the nitrite has been introduced a very little cesium chloride, a triple nitrite of cesium, copper and lead results whose solubility is much lower than that of the corresponding potassium salt, thereby increasing the delicacy of the reaction. It is possible to carry the limit of the reaction yet farther by substituting for the cesium chloride a little thallos nitrate, the test now becomes the most delicate micro-chemical test for lead which we now possess. Owing to the very low solubility of this triple nitrite of thallium, copper and lead the crystals obtained are always very small and require a high power to resolve them into black cubes and rectangular plates.

This test is a most convenient one if alloys or substances suspected of con-

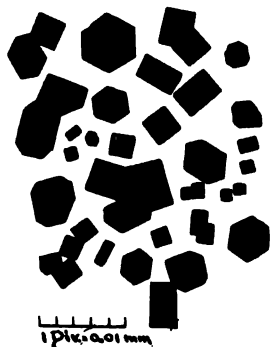


FIG. 84.

taining both lead and copper are being examined. It is then only necessary to add to the solution, sodium acetate, potassium nitrite and acetic acid. If, after waiting a reasonable time, no triple nitrite separates, cesium chloride or thallous nitrate can be added.

Nickel forms with lead an analogous salt of the composition  $[2\text{KNO}_2 \cdot \text{Ni}(\text{NO}_2)_2 \cdot \text{Pb}(\text{NO}_2)_2]$  also crystallizing in cubes, but much smaller than those of the copper salt, and yellowish brown in color instead of black.

Cobalt is immediately precipitated by potassium nitrite as a very insoluble double nitrite of potassium and cobalt in the form of a reddish brown powder, or in well defined cubes.

With potassium nitrite alone this test for lead fails in dilute solution. Careful evaporation will sometimes yield recognizable crystals.

The addition of an excessive amount of potassium nitrite is objectionable because of the fact that the triple nitrite is quite soluble in solutions of this reagent. On the other hand, it is essential that the amount added shall be very slightly in excess of that called for by theory. It is therefore necessary to proceed somewhat cautiously.

Too concentrated solutions of lead yield dense sandy black precipitates requiring recrystallization. Recrystallization can be effected by adding to the preparation a little water, a trace of acetic acid and a slight excess of potassium nitrite, then heating the preparation to boiling. Good crystals should appear on cooling.

#### *Exercises for Practice.*

Test a preparation containing Pb.

Try another preparation, this time introducing CsCl.

Try again with  $\text{TlNO}_3$ .

By a series of careful dilutions determine the limit of the precipitation of lead as the K salt, the Cs salt and Tl salt.

Test a mixture of Pb and Ni; Pb and Co; Pb and Ag, etc.

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In addition to the five tests given above, the worker must be familiar with the appearance and properties of the precipitates given by lead with such reagents as oxalic acid, primary sodium carbonate, ammonium carbonate, primary sodium tartrate, secondary sodium phosphate, potassium antimonyl tartrate, etc.

And lastly, the reduction of lead by means of powdered metallic magnesium (or zinc) must not be overlooked. By means of a little magnesium, lead can be easily separated from many complex and annoying liquids, as for example excessive amounts of the elements of Groups I and II. Moreover, the appearance of the lead precipitated in crystalline form by the magnesium is intensely interesting and very characteristic. The precipitated lead is easily separated and washed. This method is not of universal application, however, as many other metals are also reduced by metallic magnesium. The appearance of the precipitate will serve as a guide as to what metals are present.

## LABORATORY OUTLINES.

For the Elementary Study of Plant Structures and Functions from  
the Standpoint of Evolution.

## A SERIES OF FORMS TO ILLUSTRATE THE EVOLUTION OF SEX.

XVIII. *Vaucheria sessilis* Vauch. Class, Siphonæ. Order Vaucheriales. Family, Vaucheriaceæ.

This alga grows as a lax, green, felt-like layer on the surface of moist soil, and is especially common on the surface of pots in greenhouses. Other species may be found in ponds.

1. Describe the naked eye characters, noting the coarse cylindrical filaments.
2. Under low power draw an entire filament showing the branches, the tips, and the decaying part of the back end. Note the absence of transverse walls. Such a plant body is called a cœnocyte. How does the filament grow?

3. Under high power draw a short piece of a young filament, showing details—shape, vacuole, arrangement of protoplasm, chloroplasts, and oil drops. Position of chloroplasts? How can you tell that the filament is cylindrical without seeing a cross section?

4. Draw several chloroplasts. Shape? Draw some in stages of division. Describe. Look for movement of the protoplasm. Numerous nuclei are present in the cytoplasm, but these are not visible without special staining.

5. Study the sexual organs, the antheridium (spermary) and oogonium (ovary). They are usually side by side. Draw carefully and describe. Notice the septa which separate the sexual organs from the main filament. *Vaucheria* is hermaphrodite, having male and female organs on the same individual.

6. Draw the oosphere (unfertilized egg); also some spermatozoids in the antheridium. Look for free-swimming or escaping spermatozoids; also for spermatozoids entering the oogonium.

7. From the union of the two gametes an oospore is formed. Draw a ripe oospore showing the thick wall and more or less hyaline contents. Describe.

8. Contrast the two sex cells (gametes) as to size, motion, and nutrition. How is an oospore different from a zygospore? Would there be any advantage in this?

9. Special vegetative propagation by means of volvox-like colonies (compound zoospores), produced in the ends of the filaments, may be obtained as follows: Place a mass of *Vaucheria* in a porcelain dish, in water, and expose for a few days in the window until small *Vaucheria* plants are found floating on the surface. Examine very early in the morning and the volvox-like colonies may be seen escaping from the swollen ends of the filaments. In order to observe the colonies later in the morning, cover the dish, the evening before observation is to be made, so that the plants will be in absolute darkness until shortly before the material is to be studied. Study and draw. Describe in detail the formation of the compound zoospores and how they develop into new *Vaucheria*

plants. Might this process indicate some relation of the ancestors of *Vaucheria* to the Volvocaceæ?

10. Make a diagram in the notes, showing the life cycle of *Vaucheria*. (See Fig. 5.)

11. In the notes, make diagrams of the two gametes of *Sphærella*, *Paradorina*, *Eudorina*, and *Volvox*, and describe how these may indicate stages in the evolution of perfectly developed oospheres and spermatozooids.

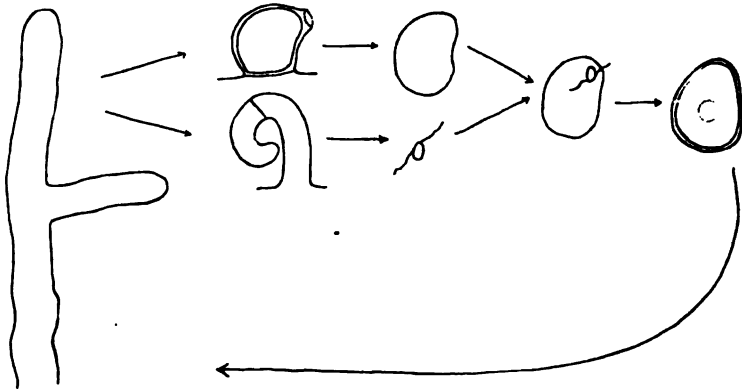


FIG. 5—Life cycle of *Vaucheria*.

12. NOTE.—The oosphere and spermatozoid are highly specialized cells, the first representing nutritive qualities, the second the active qualities. A union of the two must result in a very perfect reproductive cell. The development of sexual individuals is along the same lines as indicated in the sexual cells, and the development of one sex or the other is dependent largely on a favorable or an unfavorable food environment. Male or female sex is not an inherited quality, but depends on the environment present during the germination of the spore and the development of the embryo, and it can be directly controlled in many cases by artificial means.

#### TWO PECULIAR COENOCYTIC COLONIES.

XIX. *Pediastrum boryanum* (Turp.) Menegh. Order, Hydrodictyales. Family, Hydrodictyceæ.

This beautiful alga is found along with other species of the same genus in the sediments at the bottom of ponds, creeks, etc. It is a flat colony of cells which develop into cœnocytes.

1. Mount some of the sediment containing *Pediastrum* in water and study under high power. Draw two of the plate-shaped colonies—one with eight cœnocytes and one with sixteen. Notice the difference between the marginal cœnocytes and those in the interior. Note also the chloroplast and one or more large pyrenoids.

2. Look for colonies in which the cells in each cœnocyte have separated, preparatory to the formation of a new colony.

3. Draw a colony in which some of the cœnocytes are empty, each empty shell having a slit-like opening through which the daughter colony escaped.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Ikeda, T. Studies in the Physiological Functions of Antipodals and Related Phenomena of Fertilization in Liliaceæ. Bull. of College of Agric. Tokyo Imp. Univ. 5: 41-72, pls. 3-4, 1902.

The results of investigations on *Tricyrtis hirta* Hook., native in Japan, are recorded in this paper. Besides observations on microtome sections prepared

in the usual way, micro-chemical reactions were tested on free hand sections from fresh materials. The archesporial cell becomes the megaspore-mother-cell directly without a previous division into a primary wall cell and primary sporogenous cell. The mother cell gives rise to four megasporocytes, of which the one nearest the chalaza is functional. In the first division of the mother cell the chromatin forms tetrads strongly resembling those of such forms as *Gryllotalpa*, as described by zoölogists. Double fertilization was observed. The endosperm does not form a parietal layer, but the nuclei are evenly distributed throughout the sac. A considerable amount of endosperm is formed before the division of the fertilized egg; one of the figures showing twenty nuclei in a single section.

The chief interest in the paper lies in the micro-chemical investigation. In early stages no starch is found in the ovule, but as development proceeds, starch appears in the funiculus and outer integument and the outer and inner surfaces of the inner integument become cutinized except in the micropylar region. Starch then appears in the inner integument, though not so abundantly, and dextrine can be detected in the antipodal end of the sac. Just before the fusion of the polar nuclei, dextrine is evident in the micropylar portion of the inner integument and also in the antipodals and egg apparatus, while the starch has disappeared from the inner integument and appeared in the nucellus. After fertilization, the dextrine disappears from the integument and is present only in the fertilized egg and starch has almost entirely disappeared from the integuments and is scanty in the funiculus; but after a few divisions of the embryo, starch becomes abundant in the integuments and in the endosperm. The chalaza and conducting region have a soluble carbohydrate instead of starch.

Micro-chemical tests, together with the cytological features of the antipodal cells and the anatomical structure of the neighboring tissues, lead to the conclusion that in *Tricyrtis* the antipodals are the center of absorption of raw materials, their elaboration into the proper form and the means of transmission of food to the proper place. The dextrine in the micropylar portion of the integument is believed to serve for the nutrition of the pollen tube, while that in the egg supplies the embryo during its early stages. The cutinization of the inner integument makes it necessary for most food material from the exterior to pass through the antipodals.

C. J. C.

Schnelewind-Thies, J. Die Reduction der Chromosomenzahl und die ihr folgenden Kerntheilungen in den Embryosackmutterzellen der Angiospermen. 8vo, pp. 34, pls. 5. Jena, Gustav Fischer, 1901.

Liliaceous forms, especially *Galtonia candicans*, *Scilla sibirica* and *Tulipa Gesneriana*, were selected for this work. The reduction division in the mega-

spore-mother-cell and the divisions in the germinating megaspore are described in great detail.

In *Galtonia*, the mother cell usually gives rise to a row of four potential megaspores, but occasionally only two cells appear in the row, one of which may germinate directly to form the embryosac, a transition between the condition in *Lilium* where the megaspore-mother-cell develops directly into the embryosac and the condition where one of four megaspores produces the sac. In any case, the first mitosis in the megaspore-mother-cell shows the reduced number of chromosomes. The second division in a form like *Lilium* corresponds in all essential details with the second division in a form which is to have two or four megaspores, and there is a similar correspondence in the third divisions. In *Scilla*, it is interesting to note that while the number of chromosomes in the gametophyte is eight, the number in the sporophyte varies from eight to sixteen. In *Tulipa*, at the first mitosis in the mother cell, each of the daughter nuclei contains twelve chromosomes and in the two succeeding mitoses this number is maintained at the micropylar end of the sac, but at the chalazal end the sporophyte number may appear at the second division or the number may vary, fifteen, sixteen and twenty-one having been counted.

The writer believes that the new generation begins with the reduction division, both in the embryosac-mother-cell and in the pollen-mother-cell, and he has no doubt that the processes in ovule and in the microsporangia of Angiospermo are homologous. The four potential megaspores correspond to the four microspores (pollen grains); cases of two potential megaspores show a step toward the further reduction which is found in forms like *Lilium*. The fact that the megaspores take the form of a row, is due to position, there being no opportunity for early isolation and rounding off as in microsporangia.

C. J. C.

**Caldwell, Otis W.** A Laboratory Manual of Botany, Outlines and directions for Laboratory and Field work in Botany in Secondary Schools. 8vo. pp. vii + 107. New York, D. Appleton and Co., 1902.

Part I considers plants from the viewpoint of their physiology and ecology, while Part II considers their structure and evolution. A year's work is outlined.

The directions for securing material, the outlines for study and the suggestive questions should prove useful to the busy teacher.

C. J. C.

**Nelson, Aven.** An Analytical Key to Some of the Commoner Flowering Plants of the Rocky Mountain Region. 8vo. pp. vii + 94. New York, D. Appleton and Co., 1902.

This booklet is intended as an introduction to a study of a manual rather than as a substitute for one. The list includes the more useful laboratory

material in the region from Montana to New Mexico with a somewhat variable eastward and westward extension. In general, the sequence of families is that given in Engler and Prantl's *Die Natürlichen Pflanzenfamilien*.

C. J. C.

**Hegelmaier, F.** Ueber einen neuen Fall von habitueller Polyembryonie. Ber. d. deutsch. bot. Gesell. 19: 488-499, 1901.

The writer finds that in about two-thirds of the half ripe seeds of *Euphorbia dulcis* Jacq. there is more than

one embryo, the number ranging from two to nine. In the ripe seed the number is smaller, usually two or three, and one of these considerably larger than the others. The embryo which develops from the egg is the only one which has a suspensor and is also the one which becomes the largest embryo in the ripe seed. Of the other embryos, some come from synergids and some from the cells of the nucellus. A large per cent. of the polyembryonic seeds is not capable of germinating on account of the disorganization of the embryo.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

**Morgan, T. H.** The Disposability of Gravity in the Development of the Toad's Egg. *Anat. Anz.* 21 : 313-316, 1902.

Roux's experiments on the frog's egg apparently showed gravity acting in a definite plane not to be necessary for

the development of the egg. Other authors have disagreed with these results, but the present work confirms them as to the toad's egg. A rubber tube connected the water tap with a long glass tube, the latter was made to reach nearly to the bottom of a glass jar (450 x 50 c.). Water turned on carried the mass of eggs round in a most irregular way, rotating them once in 5 to 15 seconds. The toad was killed and eggs with sperm put in small jars that were kept stirred, rapidly and irregularly, for half an hour. Then they were put into the jars described above. The cleavage of such eggs removed in the two-cell stage (after 5 hours) was normal, also after 25, 36 and 48 hours. Those removed after several days' rotation also produced normal embryos. From these observations it is concluded that gravity need not be a determining factor in the development of a bilateral plane in the apparently radically symmetrical egg.

A. M. C.

**Rosner, A.** Monochorial Twins. *Bull. Internat. Acad. Sci. Cracovie*, 8 : 443-450, pl. 1, 1901. (Review in *Jour. Roy. Mic. Soc.* 159-160, 1902.)

After discussing the numerous theories of monochorial twins in man, the author concludes that they arise from a

bi-ovular Graafian follicle; the two ova, liberated together and normally fertilized, are united together by some cells of the discus proligerus and become surrounded by a common caduca. The chorionic areas which are in contact and form a partition between the two developing ova may disappear simply from malnutrition. Rosner studied animals habitually producing monochorial twins or rather multiple foetuses. The two armadillos, *Praopus hybridus* and *Dasytus novemcinctus*, were chosen. The first usually has eight foetuses, always of the same sex and in a common chorion; the second has four under similar conditions. Sectioning ovaries of *Dasytus*, Rosner found many of the Graafian follicles to be pluri-ovular. Out of 52 follicles, 22 contained more than one ovum; of these, 11 had 2, 7 had 3, 2 had 4, 1 had 5, and 1 had 7. The primordial follicles in the ovary of *Dasytus*, foetal or adult, contain but one ovum and Rosner shows that several of these fuse to form the pluri-ovular follicle; each ovum is normally fertilized, each develops a chorion of its own, but within the investments of the caduca the chorionic partitions are dissolved till but one chorion remains. That the mono-chorial foetuses are always of the same sex is interpreted as due to similarity of environment from the Graafian follicle onwards.

A. M. C.

Beard, J. The Numerical Law of Germ Cells.

Anat. Anz. 21: 189-200, 1902.

There are, broadly speaking, two alternatives as to the relationship of a set of germ cells and an embryo: 1. The

embryo may have formed the germ cells. 2. The germ cells existed first and the embryo arose from one or more of them. Hence the embryo is a development of one primary germ cell for the purpose of guarding a certain set of germ cells during a portion of their life cycle.

The enumeration of germ cells gives added importance to the latter point of view. It is soon seen that the number is a constant for the individuals of any species, and it varies with different species. This number may be called  $2^n$ . There is often a disparity between their arrangement in the two halves of the body, also in the total number of normally placed functional germ cells. In *Raja batis* (the form especially studied), the germ cells must migrate into the embryo. This causes a certain amount of irregularity in distribution. By rearing many thousands of Elasmobranch embryos the writer tested this point. Surprisingly few abnormalities occurred, but these few on examination showed many interesting peculiarities in the distribution and number of germ cells. This is probably a consequence from, not a cause of, the abnormality. A summary of five embryos as to normally placed germ cells is as follows:

No. of Embryo.	Left Side.	Right Side.
742	None	Normal
743	Normal	Few
744	1	None
745	Normal	4
746	14	46

There is great irregularity in distribution, in some cases the germ cells are present in the defective side of the body and absent in the apparently normal one; in some the cells are all abnormally placed and in others a few in each case. The aberrant cells occur in all places. They are some of them very large, the megaspheres, equivalents of 8, 4, or usually 2 germ cells.

Enumeration of the germ cells in embryos of *Raja batis* showed two numbers near which the total always came. They were 512 or half of it, 256. These are shown to correspond to the two sexes, the more numerous the female, the less the male.

The following forms were also investigated and  $2^n$  being assumed as representing the number, the actual figures are shown to vary from 8 to 512 in the different animals: *Rana esculenta* 8, *Petromyzon planeri* 32, *Acanthias vulgaris* 64, *Scyllium canicula* 128, *Raja batis* 256, *Raja batis* 512. Thus all powers of 2 from  $2^3$  to  $2^9$  excepting  $2^4$  are seen. From this total must be deducted that number of cells that goes to form the embryo. This makes the formula for any given Metazoan to be  $2^n - 1$ . It should be borne in mind that the two symbols  $2^n$  and  $2^n - 1$  have recently been used in a different but equally significant sense by the late Gregor Mendel.

A. M. C.

## CURRENT ZOÖLOGICAL LITERATURE.

CHARLES A. KOFOID, University of California.

Books and Separates of Papers on Zoölogical Subjects should be Sent for Review to Charles A. Kofoid, University of California, Berkeley, California.

**Prowazek, S.** Zur Entwicklung der Gegarinen. Arch. f. Protistenkunde, 1: 299-305, Taf. 9, 1902.

The gegarines *Monocystis agilis* and *M. magna* were obtained from the testis of the earthworm. The testis was dissected out and fixed in corrosive-acetic. Sections were stained in hæmatoxylin-rubin, in Ehrlich's triacid stain, and in iron hæmatoxylin and orange G. In Ehrlich's triacid the protoplasm of the sporozoite stains red and the nucleus bluish green. Fine nuclear details are best secured with the iron hæmatoxylin. Cover-glass preparations by the Ehrlich method were also stained in the triacid stain with differential results. Romanowski's method gave no satisfactory preparations of this material.

C. A. K.

**Goodrich, E. S.** On the Structure of the Excretory Organs of *Amphioxus*. Quart. Journ. Mic. Sci. 45: 493-503, pl. 27, 1902.

The "kidney" of *Amphioxus* may be demonstrated when living material is at hand. The specimen is pinned out on its back in a shallow dish of sea water. The atrium is ripped up with a needle along the mid-ventral line and the two metapleural folds pinned aside. The exposed pharynx is then also ripped up with a needle and portions of the right or left side of the pharynx can then be torn out with the forceps and placed on a slide, care being taken to lay the outer side uppermost. Permanent whole mounts or sections may be made of these parts by the usual methods. Details of structure in the fresh material must be studied with oil-immersion and high eye-pieces. The author concludes that the so-called kidney of *Amphioxus* does not open into the cœlom by cœlomic funnels, as described by Boveri. The so-called thread cells resemble the solenocytes of annelids, in fact the whole organ is much like the nephridium of some annelids, for example of the Glyceridæ.

C. A. K.

**Miura, K.** Amœbenbefund in der Punctionsflüssigkeit bei Tumoren der Peritonealhöhle. Mitth. Med. Fac. Kais. Jap. Univers. 5: 15 pp., 1900.

*Amœba miurai* Ijima has been found in three cases of peritoneal tumors in man in the fluid exudate drawn from the body cavity. Upon standing the fluid separates into a yellowish upper layer and dark red lower one in which are white and red blood corpuscles, fibrin clots, tumor cells, and the structures regarded as amœbæ. The fluid was drawn with aseptic precautions and kept at the temperature of the body during examination under the microscope by means of a warming apparatus. Nuclei were demonstrated by acetic acid and preparations made by drying the fluid upon the slide and staining in hæmatoxylin. The consistency of the organisms is greater than that in most amœbæ and movements are very slow. They affect principally the villi on the knoblike protuberance and sometimes take the form of wave-like changes in the surface. Many moribund and disintegrating individuals occur with the living ones. This *Amœba* is reported in the exudate associated with tumors of three very different types.

C. A. K.

quantities of  $\alpha$ -naphthol ( $C_{10}H_7OH$ ) and para-diamido-benzene (or para-phenylene diamine:  $C_6H_4(NH_2)_2$ ), in equimolecular proportions. This solution, at first colorless, becomes deeply tinted on oxidation. 2. Oxidation of dimethyl p-diamido-benzene with  $\alpha$ -naphthol; result is an intense greenish-blue dye. 3. Formation of the dimethyl derivative of quinone anilimide by oxidation of phenol with dimethyl p-diamido-benzene. Experiments were made on the following organs and tissues of the frog: Liver, spleen, pancreas, lung, kidney, testis, ovary, various parts of the intestinal tract, striated muscle, parts of the nervous system, the thymus, etc. The method was to submit thin slices of the tissue (either fresh or alcoholic) to the action of the indicators until they were well impregnated with the colored oxidation products. The solutions of the indicators were made either in normal saline solution (for fresh tissue) or in distilled water (for alcoholic material).

The experiments showed that, in general, oxidation takes place most rapidly in those organs and parts of organs where nuclei are most numerous and most densely aggregated. In the case of blood corpuscles (and also in some other cells) it could be seen that the oxidation products were deposited chiefly in and about the nucleus, especially at the surface of contact between nucleus and cytoplasm. The general conclusion is that "the nucleus is the chief agency in the intracellular activation of oxygen."

R. P.

Moszkowski, M. Ueber den Einfluss der Schwerkraft auf die Entstehung und Erhaltung der bilateralen Symmetrie der Froscheies. Arch. f. mikr. Anat. Bd. 60, pp. 17-65, 1902.

Moszkowski concludes after a thorough, critical examination of the literature, and a series of observations and experiments of his own that the action of

gravity is a necessary factor for the development of the frog's egg. The part played by this force is the determination of the symmetry plane of the egg, and therewith the median plane of the future embryo. This result is brought about by a rearrangement of the contents of the egg (sinking of the white yolk along the periphery, etc.) which takes place after the egg has been laid. While the egg is within the body it occupies all possible positions with reference to the direction of gravity, but the molecular cohesion of the yolk is so great that no change in the relative position of yolk and other material takes place. After the eggs are laid water is absorbed and the consistency of the egg substance is thereby lowered. This lessening of the consistency occurs before the surrounding gelatinous envelopes have swollen enough to allow free rotation of the egg as a whole, and consequently a flowing of the egg substance takes place, bringing the heaviest constituents to the lowest point in the egg. In the case of the unfertilized egg five to six hours are required for this process to take place, while in the fertilized egg it is completed in, from one-half to three-quarters of an hour.

If the egg in the two-celled stage is reversed (i. e., placed with the white pole uppermost), and held in position by compression, not only is the symmetry of the whole egg destroyed, but frequently new symmetry planes are formed in each of the blastomeres. In these cases each of the first two blastomeres gives rise to a whole embryo, whose organs are symmetrically arranged about the new symmetry plane. By treating the egg in the four-celled stage in the same way it is not possible to produce four embryos, but instead a single symmetrical embryo develops. Beyond the four-celled stage the egg, when placed in an abnormal position, has no means of protecting itself from the harmful influence of gravitation.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Glogner.** Ueber Framboesia und ähnliche Erkrankungen in den Tropen. Virchow's Archiv., Bd. 168: 443-456, 1902.

Interest in tropical diseases should kindle in us a desire to know something concerning the pathology of

Framboesia or Yaws. It and other allied diseases have had a number of investigators. Among them is Dr. Glogner, who obtained while in the Dutch West Indies six Framboesia warts or tumors from four patients aged 20, 5, and 18 years, and 15 months respectively. The specimens were hardened in Müller's fluid and embedded in paraffin. The sections were cut and studied at the Pathological Institute in Berlin.

By Framboesia we understand a skin affection lasting usually over a month, rarely over a year. It is generally accompanied by no prodromata, but at times fever and neuralgia are noted. The prognosis is favorable. Children are more prone to it than adults. The eruption begins as round papules which are more likely to appear on the face, especially about the mouth and nasal cavities. These papules gradually increase in size and, joining with others, become confluent and form excrescences or tumors, which project over the surrounding skin. The epidermis of these tumors subsequently become necrotic and crusts are formed. These tumors heal and leave no scars, but merely a very noticeable mark.

If secondary infection has occurred ulcers are formed, which healing ultimately leave a scar. Verrugas Peruviana and Bubas are so unlike Framboesia that it is best to consider them separate and distinct diseases until more work is done on them.

Charlouis, Cornil and Renaud have worked especially on the pathology of Framboesia. The process in these tumors is generally considered to be a chronic dermatitis or a granuloma. Cornil and Renaud, however, consider the tumors to represent sarcoma tissue in different stages of development. Glogner noted a proliferation of the connective tissue cells of the cutis, and of the endothelial cells of the lymph vessels as the most prominent characteristic of the lesion. Next a moderate increase in the epidermal epithelial cells, and finally a migration of leucocytes into the tissues surrounding the blood vessels. The migration was evidently due to mechanical or chemical injuries to the tissue or to the influence of bacteria, as it was not seen in the smaller tumors or papules. Glogner, also, thinks these tumors belong to Virchow's group of granulomata. He found no plasma cells, but mast cells were present in considerable numbers. Some sections showed giant cells and beginning degenerative changes in some of the cell's nuclei. He noted a leucocytosis in all of his cases due to an increased number of lymphocytes. The average percentage of the polynuclear leucocytes was 62.5 in the eight cases he examined, while the mononuclear forms amounted to 38.8. The lesions in Verruga are those of an acute inflammatory infiltration. In Bubas infiltration of the papillæ has been found by one observer with an increase in the connective tissue. Plasma cells were also observed and scar formation resulted in all of the cases.

Glogner is unable to explain the cause of Frambœsia. Many sections were stained for bacteria, and countless methods were employed—all with negative results, however. Blastomyces were in vain looked for with special staining reactions. Pierce, Nicholis and Watts, however, have described and cultivated bacteria as the causal agents of this disease. By inoculation Lange, Morris and Steele obtained negative results, but Charlouis, though successful in causing some lesion, was unable to reproduce the disease. W. R. S.

**Brault.** Le Glycogène Hépatique dans les Cirrhoses. *Archiv. de Med. Experiment. et D'Anat. Pathol.*, 14: 453-469, 1902.

Contrary to the expectation that the glycogenic function of the liver would be suspended in cirrhosis, Brault has found that it is well maintained. He gives the clinical histories, with the autopsy findings, of five cases. In three of these tuberculosis was a complication, in one the cirrhosis was latent, the patient dying of pneumonia, while in another death was due to the rupture of some œsophageal varices. The autopsies were performed from three to thirty-six hours after death, and in all of them the liver was found to possess a great number of cells, intact or fatty, containing glycogen. This reaction was obtainable even in fragments of lobules that were entirely isolated by surrounding connective tissue. The glycogen was never uniformly shared by all the cells in the lobule. In one case the cells in the center of the lobule were fatty, while those in the intermediary zone contained both fat droplets and glycogen. In the peripheral zone or peri-portal region the glycogen was found in the greatest amount. In another case the reaction was obtained after an antiseptic fluid had been injected into the peritoneal cavity to prevent putrefaction.

Brault concludes that the pressure exerted by connective tissue on isolated cells has no effect on their glycogenic function; that it makes no difference in the finding of glycogen whether the cells are fatty or not, or whether they have their usual dimensions or are decreased in volume. These cells may be found in lobules or isolated and apart by themselves. He inclines more to the view that the presence of glycogen in cirrhosis is supplemental and vicarious rather than physiological. Its excessive formation in this condition is due to some nutritive excitation, and for a certain number of cells is only temporary, as these cells subsequently undergo degenerative changes and disappear. W. R. S.

**Drake.** Trichinosis. *J. of Med. Research*, 8: 255-267, 1902.

Since Brown first noted the presence of an eosinophilia in Trichinosis it has been an important diagnostic symptom in over twenty-five cases. The only exception has been Da Costa's case, reported a year ago. Its absence in the case may have been due, as Coplin pointed out, to the fact that the patient was not seen in the original attack but in a recrudescence of the disease.

Drake did differential blood counts on 15 trichinous and 15 non-trichinous swine. He found a varying percentage of eosinophiles, which, on the average, was one per cent. lower in the trichinous than the non-trichinous swine. He then relates the clinical histories of four cases of trichinosis which showed no eosinophilia. In each case a portion of muscle had been excised and trichinae found therein. In his conclusions he states that eosinophilia may not be present during the course and recrudescences of the disease and that its absence by no means eliminates the possibility of trichinosis being present. W. R. S.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

**Ernst.** Ueber den Bau der Bakterien. Cent. f. Bac. II, 8: 1, 1902. The so-called Babes-Ernst bodies in bacteria have become widely known

and much studied, although there has been no unanimity in regard to their significance. Ernst, after several years' silence upon the subject, now contributes the result of a long series of new studies upon these bodies which bear his name. Most of the paper is taken up by a description of the appearance of these bodies as shown by recent studies. His method of study is the intra vitam staining which has recently come into use. For a stain he uses grains of neutral red and methylen blue which are allowed to dissolve slowly in the liquid in which the bacteria are living and stain the organisms. The observations which he makes are many of them novel, and an examination of the two plates which accompany the paper is necessary to give any idea of the wonderful variety which these bodies present when studied by this method. One of the most interesting facts pointed out is that these bodies are frequently in constant motion circulating around within the bacterium. In regard to their significance Ernst takes no very positive ground. He doubts that they are homologous with nuclei as some have believed, and is even uncertain whether to look upon them as vital parts of the bacteria body. Their meaning he regards as still wholly hypothetical.

H. W. C.

**Gauss.** Babes-Ernst'sche Körperchen und Virulenz bei Bakterien. Cent. f. Bac. I, 31: 92. This author has attempted to test the truth of the conclusions recently reached by Marx and Woihte that the

pathogenic power of the bacteria can be determined by the presence of so-called Babes-Ernst bodies. These authors have reached the conclusion that if these bodies are present in the bacteria the organisms have pathogenic properties, whereas if they are absent the pathogenic properties are also absent. The practical value of such a conclusion, if true, is very considerable, because it would enable the microscopist quite readily to determine whether or not a given culture of bacteria possesses pathogenic properties. Gauss was very doubtful as to any wide application of this law, and for the purpose of demonstrating its truth or falsity has made a series of studies with *B. pyocyaneus*, which he isolated from an acute case of pneumonia. The cultures which he obtained were carefully tested as to their pathogenic power in animals, and he was able beyond question to demonstrate that the organisms were endowed with strictly pathogenic properties, but no Babes-Ernst bodies were found. His method of examination for these bodies is given in detail and was the method ordinarily used by microscopists. The bodies appear to be totally absent, and yet the bacteria in question were strictly pathogenic. The author is, therefore, inclined to believe that the law advanced by Marx and Woihte is not a strict one and can never be used practically for determining pathogenic properties of bacteria. H. W. C.

**Fedorowitsch.** Ueber die Körnigkeit der Bakterien. Cent. f. Bac. u. Par. II, 8: 481, 1902.

The method adopted by this author for study of the internal structure of bacteria is as follows:

The bacteria are very carefully spread out upon a cover slip and treated for 5-10 minutes with saturated anilin water-gentian solution, washed quickly with water and then treated for one minute with Gram's iodine solution, and again washed in water. They are then treated for 1-2 minutes with one-half or one per cent. safranin water solution, and a third time washed in water. They are then dried and treated with a mixture of anilin oil and xylol until properly decolorized. By this means the contents are differentiated, parts being colored strong violet, while the greater part of the body is colored a rose color. Some excellent figures show the results. He finds that spore bearing bacteria have a peripheral row of granules which become spores; while non-spore bearing bacteria show similar granules which do not form spores.

H. W. C.

**Moore.** The Isolation of the Typhoid Bacillus. British Medical Journal, p. 703, 1902.

The author has given a contribution to the much worked subject of the separation of the typhoid and colon bacillus.

He gives two methods, one of which is dependent upon the motile property of the typhoid bacillus, but which he finds not to be practical. The second, which he thinks is very useful, depends upon the use of Elsner medium, which he modifies by the substitution of agar for gelatin. The method is as follows:

**Modified Elsner agar medium.** Take 500 grams of potatoes, peel them and scrape them on a grater. The pulpy mass so obtained is then macerated in a liter of water for 3 or 4 hours; strained and allowed to stand over night. The next morning the clear supernatant liquid is decanted and the volume made up to a liter. This liquid is rendered alkaline and 20 grams of agar added. The process is then continued as for ordinary agar. When sterile the medium is distributed in test tubes, and immediately before using .5 c. c. of the following solution is added to each tube:

Potassium iodine	-	-	-	-	10 grams.
Water	-	-	-	-	50 c. c.

The medium should be used fresh.

Plates prepared with this medium and sown with mixtures of typhoid and colon bacilli are incubated for 24 hours at 37°. At this time an examination with a low power will show an evident and characteristic distinction between the typhoid and colon bacilli. By the use of this method he is not only able to separate the two organisms in artificial mixtures, but has been able to find the typhoid bacillus in cockles which were suspected as having been the cause of the outbreak of typhoid fever.

H. W. C.

**Bariekow.** Beitrage zur Differentialdiagnose des Typhus bacillus. Wien. Klin. Rund. No. 44, 1901.

The author uses one culture medium composed of peptone or nutrose 1 per cent., milk sugar 1 per cent., NaCl 5

per cent., distilled water 100 c. c., and a second in which grape sugar is substituted for the milk sugar. In 24 hours' growth the coli bacillus shows a strong acid reaction upon both media, but grows more rapidly upon the second, while the typhoid bacillus produces acid in the second but not in the first medium. The author always succeeds in differentiating the two in 24 hours.

H. W. C.

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## The Museum.

The museum has become so well established in our cities, and is so readily recognized as one of the important factors in popular education, that it is unnecessary to dwell upon its usefulness. Its development and its construction, both as regards mere mechanical features and educational influence, has not been in all instances equally well advanced, and to-day a complete inspection of all the museums of a country would readily afford a classification as diverse in its elements as the Midway Plaisance at a country fair, and that ideal product of administration in the great cities where investigation goes hand in hand with recreation.

It seems possible to reduce to a system of approximate exactitude, methods of installation, methods of administration, arrangement of exhibits and, within the limits of moderate artistic variation, architectural design. Perhaps this is a false end to propose. Formal monotony might be urged as a formidable objection to any such scheme, but if the museum at the outset is fundamentally an *educational* instrumentality, it is not difficult to prove that educational methods of the best type should be very nearly identical. Museums should not, it can be insisted, afford limitless chances for the vagaries of exhibitors, but must defer to some establishment of opinion and use intended to secure the best educational results.

The tendency of educational movements to-day, if the impressions left after reading the papers and addresses of educators are not misleading, is to bring about comparative uniformity in systems and, of course, the fundamental assumption is that the human mind is quite similarly constructed and that in its elements all education follows quite similar methods. And so, a uniform museum system can be frankly regarded as desirable, and that such means of education as are proven best in New York and London may also be expected to work the same results in Chillicothe or Cañon City. And a system may be elaborated quite independently of financial equality. The wealth of New York can be justly

expected to provide a more expensive illustration of the system than Cañon City, but the principles of the system in both places can be identical.

At the outset the museum system consists of the *physical constants*, the *educational aim* and the *government*, the administration. The *physical constants* are: (1) The Museum Building, (2) the Museum Hall, (3) the Museum Case. It is the physical constants that naturally underlie all later development in the museum, and they are the most obvious and useful subjects which can be discussed.

#### THE MUSEUM BUILDING.

The museum building must meet these requirements: those of space, of light, and of arrangement. It should be large enough to accommodate the objects intended for exhibition; it should admit of complete illumination, and the maximum available at the darker hours of the day and of the year; it should be so

FIG 1.—Natural History Museum, London.

built that the departments represented maintain the necessary separation, and permit in each a rational relation of parts.

The treatment of the museum building is epitomized in the *Exterior* (and environment) and *Interior*. Environment, as suggested in the parenthesis, demands thoughtful consideration. It can be, for cogency and brevity, be placed under the topic of the Exterior.

The position of a museum involves important consequences in the large cities. It should be accessible from all quarters of a community, it should be placed where least likely to incur accident or destruction, it is preferably built upon an elevation than in a hollow, its surroundings should minister to the pleasure of the eye and involve an expression of distinction and beauty.

As one of the deteriorating agencies, accompanied sometimes with very serious losses of time, money, patience and material, is dust, a position as free as

possible from exposure to it should be chosen. On this account, and because their surroundings have an æsthetic value which enhances the interest of the museum building, and imparts also the pleasurable stimulus of expectation,

FIG. 2.—Art Galleries and Museum, Glasgow.

museums may well be placed in public parks, squares and gardens. Walks, parterres, diversified approaches, and even the use of symbolic statuary could be elaborated, most helpfully, but usually the relinquishment of such designs is imperative from lack of room or money. As in all efforts at embellishment, effects, unless delicately and effectively designed, are either overdone or miss the mark through insipidity, perhaps it is better to omit such accessories.

FIG. 3.—Science and Art Museum, Dublin.

The museum building, in its exterior, furnishes, of course, room for some latitude in design and ornamentation. It is a proper principle, however, in architecture that types of buildings, denoting separate and exclusive uses, having been

historically evolved, the museum building should also, if possible, express its purpose to the eye. In a generalized way, a vertical surface of wood or stone, pierced by a succession of windows, too extended or too large to be construed as intended for domestic illumination, and unnecessary in a court house, an armory, a state house, a store, or a theatre, and given some distinction by details of con-

FIG. 4.—American Museum of Natural History.

struction, would usually be interpreted as a museum of nature or industry. A lower building with ample skylights and a probably more ornate treatment, would be regarded as a museum of art. Numerous exceptions to any such generalized expressions occur to everyone, but no matter to what extent the department store or the concert hall, libraries, or university buildings imitate one or the other, the idea of a more or less long, rectangular space with abundance of light entering

FIG. 5.—Provincial Museum, Hanover.

from the sides, and the idea of a similar space with light descending into it from above, are associated properly with the museum building. That such a design obtains in fairs, expositions, crystal palaces, cattle shows, and menageries, does not disturb this propriety, as all such exhibits are in the nature of temporary or perverted museums.

Such a nucleal box in its base simplicity is exemplified in ordinary town constructions, but when this box is united with other similar units, when extension on all sides and super-imposition as well occur, then the architectural severity or monotony requires modification, and at this juncture the taste, ingenuity, and invention of the architect enters, to confer upon the composite mass dignity and charm. The architect seems at times superserviceable in this respect, and illustrations are not wanting of his neglect of the real functions of the museum, and their subordination to the architectural complexity or splendor of the building. Still the architect's skill and cultivated eye are essential, and a noble museum building in the large cities should be imperatively insisted on. Anyone who has

FIG. 6.—Art Museum, New York.

seen the Kensington Museum (Fig. 1) has felt the influence of its beauty, external nobility, and elegant composition, though even in that instance architectural elaboration may have slightly encroached upon museum claims. On the other hand, the design of the museum in Glasgow, while forming an impressive and varied architectural spectacle, appears too curiously and intricately developed into towers and finials (Fig. 2), and its windows too much broken up by transoms or mullions. The natural history section of the Dublin Museum for science and art is an example of the *box* unornamented or changed, and while its solidity is unmistakable, if it stood alone, its blankness and gloom would prove repellent (Fig. 3).

The psychological influence of a beautiful building is not to be lightly considered. Museum curators are themselves at times too indifferent to these influences, and, immersed in the scientific interest or rarity of collections of specimens, fail to realize how the *receptivity* of the common public is stimulated by environmental charm.

The architectural marking of the *box* can be effected in three ways: (1) By the successional relief of the facade by towers, columns, square or round, as unity of design requires; (2) by surrounding the *box* by colonnades, or so combining the box units as to form irregular re-entering or projecting surfaces; or (3) by simple embellishment.

The first device is exemplified in the Kensington Museum, the American Museum of Natural History (Fig. 4), the Provincial Museum, Hanover (Fig. 5), the Vienna Hof. Museum, etc.; the second, in the National Library, Dublin, the National Museum, Washington, in a measure in the Art Museum of New York, etc.; the third (combined with the first), in the Corporation Museum and Art Gallery of Glasgow, and also in the Art Museum of New York (Fig. 6).

American Museum of Natural History.

L. P. GRATACAP.

### Sectioning Fresh Plant Tissues.

In histological studies of leaves and other thin plant tissues, it is often very desirable to make sections, serial or otherwise, which have never been injured through chemical processes of hardening and embedding nor by the heat to which the tissues are exposed in paraffin embedding. Free hand work is often satisfactory, but by this method the sections of a series are apt to vary greatly both in thickness and in the angles at which they are cut. Where greater uniformity is desired, the writer has practiced a system of rapid and efficient embedding which may prove useful to other workers.

For embedding a small piece of leaf or other like tissue, two pieces of paraffin are used, each piece being about 20 mm. long, 14 mm. wide, and 3 mm. thick. The portion of leaf to be embedded is properly placed near the center on one piece of paraffin and the other piece is firmly pressed above it in such a manner that the margins of the two pieces of paraffin coincide and are pressed firmly together. A heated scalpel is now run entirely around the edges of the paraffin blocks so as to firmly melt them together where touched. The entire cake, containing the centrally embedded square of leaf tissue, is then thrown into cold water until sufficiently firm to be fixed upon the microtome plate and trimmed for sectioning in the usual manner. Where paraffin is of the right degree of hardness, is held at the proper temperature, and the other conditions are as they should be in all microtome work, it is not difficult to obtain uniform sections of five microns thickness and of perfectly fresh plant tissues.

I usually keep a box filled with suitable paraffin pieces, so that a number of squares of leaf tissue may be embedded at one time, stiffened in water, placed in the microtome, and sectioned in a fresh and uninjured state in a few minutes

time. By throwing the sections into cold water, the plant tissue usually separates readily from the hardened paraffin and may be examined at pleasure.

In making the paraffin blocks, a plate of heavy glass is selected which is about one foot square. Around the margin of this glass plate, on one surface, are cemented strips of glass one inch wide and one-eighth of an inch thick. Melted paraffin, of the proper degree of hardness for sectioning work, is then poured upon the leveled plate until it floods the plate to the top of the marginal glass strips—i. e., until the paraffin is one-eighth of an inch thick over the entire surface of the plate. When sufficiently cool, the paraffin is marked into checks of any required size with the point of a scalpel, so that the entire sheet of paraffin will readily break into squares of the proper size when cold. A ruler is used in checking off the surface. If one of the marginal strips of glass is left so that it can be removed when the paraffin is cold, the latter may be more readily freed from the glass.

NEWTON B. PIERCE.

U. S. Depart. of Agriculture, Pacific Coast Laboratory and Gardens, Santa Ana, Cal.

## Collecting and Preserving Fungi.

### UREDINEAE.

The methods used to prepare first-class specimens of the flowering plants are mostly applicable in collecting the Uredineæ. The same care in changing driers is needed to produce attractive specimens. The best method is to use a portfolio or press in the field, and to arrange the specimens as soon as collected. Only a few driers should be carried, as the specimens will keep for a day in the sheets of thin paper.

Where it is windy or large quantities are to be collected a botanical box is convenient to carry specimens for a short time. Many grass leaves will curl if not attended to very soon after gathering. I prefer to cut the leaves of grasses and sedges into four-inch pieces when collected, and to discard those not much attacked by the rust. Pieces of this length are more readily kept flat. The ligules of grasses, and portions of the inflorescence of both grasses and sedges should be included whenever possible so that the determination of the host plant may be verified. Great care should be taken in collecting to find the uredo and æcidial stages, as in many cases the uredosori offer much better distinctive characters than the teleutosori. The æcidia should be collected when the cups have opened, and before they are so old that the borders and spores have mostly disappeared. As they are often somewhat fragile, better specimens can be made by applying only sufficient pressure to keep the leaves flat. In all cases the host should be *accurately* determined. Many serious errors have been made on account of carelessness in this. In some cases the teleutospores are to be found on the stems, when uredospores only occur on the leaves. This is particularly true with the species of *Phragmidia* on *Potentilla*, early in the season.

I think that there is only one method of arranging the specimens in a collection of any size, and that is according to the host plants. I use the standard herbarium sheets and enclose the specimens in packets which are fastened to

the sheets with the ordinary photographic paste. This permits the packets to be removed without tearing the paper. The different genera of the Uredineæ are kept on separate sheets, and only those on one species of host kept on a sheet. I am arranging the hosts according to Torre and Harms' *Genera Siphonogamarum*, as that work appears, and each sheet is numbered at the top with the genus number used therein. This facilitates the keeping of the specimens in order. Alphabetical indexes of the genera of the larger orders are pasted on the doors of the herbarium cases. The packets are placed far enough apart to permit of adding illustrations, references to literature, etc. The packets are the ordinary ones furnished by the Cambridge Botanical Supply Co., not the specially cut form. A most important aid in the study of the Uredineæ is a collection of mounted slides. I use a method devised by Prof. J. C. Arthur, which is to mount the spores dry, fastening the cover-glass with a narrow strip of gummed paper. I number the slides, and on the packet from which the mount was made is marked "Slide No. —."

These slides save a large amount of valuable herbarium material, are always ready to examine dry to show the surface markings of the spores, and by adding a drop of distilled water, the spores soon swell to their full size.

In this arrangement of a herbarium no time is lost in looking up names of fungi to see what the collection contains (which might require looking up various synonyms), and unnamed specimens can be placed in the collection and they will be in the proper place when the fungi on that genus or order are being studied. Changes in the name of the fungus do not affect the position of the specimen in the herbarium.

E. W. D. HOLWAY.

Decorah, Iowa.

## New Pathological and Bacteriological Laboratories of the Medico-Chirurgical College, Philadelphia.

In planning the new laboratories for the Medico-Chirurgical College, a successful attempt has been made to conduct all of the work of a department upon a single floor of a large and well lighted building, securing for those departments where the microscope is to be used the upper and best lighted stories. The pathological and bacteriological laboratories were, therefore, placed upon the fourth floor of a tall building with an unobstructed view from the windows of every side.

The Department of Pathology and Bacteriology is divided into two main laboratories, one for the teaching of each subject. Adjoining each are two small rooms, one the private apartment of the demonstrator or instructor for that department, the other a stock-room for the supplies and preparations used in teaching. A morbid anatomy cabinet also adjoins the pathological laboratory. There are also a room for advanced students who desire to do original and individual work, a small lecture room provided with an autopsy table with alberene top, central drain and water supply, convenient sink, electric light,

blackboards, projecting screens, etc., a dark room for photographic work, a janitor's room, and a suite of apartments for the professor.

The large windows of the pathological laboratory, each nearly six feet wide, and five in number, face the north, and to facilitate morbid anatomy demonstrations very large tables project from each window into the laboratory, the men sitting on both sides of these tables facing one another, and receiving the light from the side. Five such tables divide the class into groups of ten men each, and morbid specimens can be presented to each group from the end of the table. Between the tables, and also between the windows against the walls, there are sinks and microscope lockers. On the opposite side of the room,

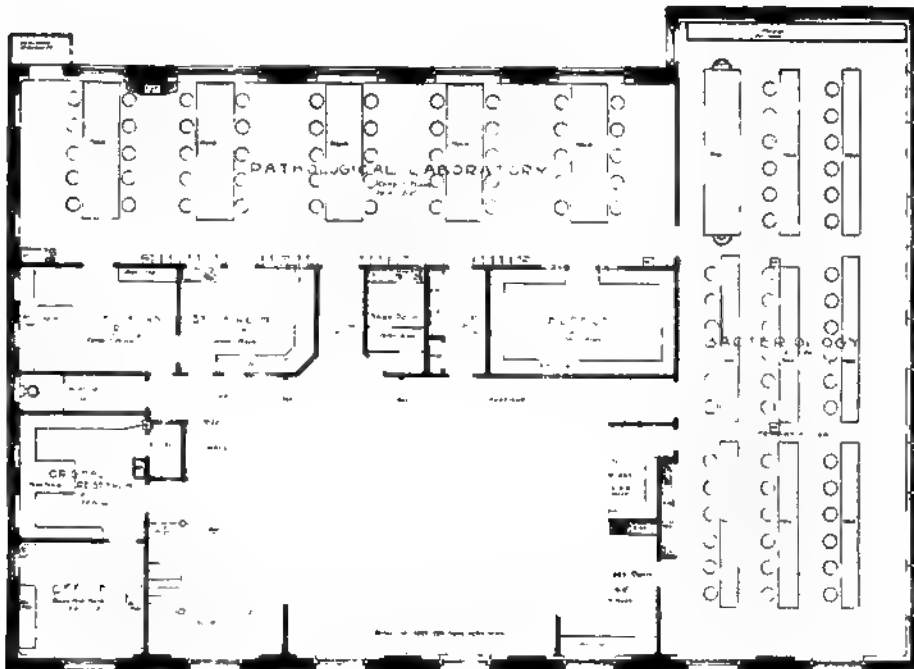


FIG. 1.—Fourth floor plan. Prof. McFarland's Laboratories. New Laboratory Building of the Medico-Chirurgical Hospital, Philadelphia, Pa.

lockers are provided for the instruments, slide-boxes, reagents, notebooks, etc., of each man in the class. Each locker measures 12 x 14 x 18 inches, has one shelf, and contains a moveable tray in which staining dishes and reagent bottles can be kept. The method of laboratory teaching provides that each man shall take charge of his own reagents, returning them to his own locker at the end of each demonstration, and returning the microscope to its locker, thus leaving the room unincumbered, so that it can be kept tidy and clean with comparatively little effort.

In the laboratory of bacteriology a more conventional arrangement of tables has been adopted, the men sitting at narrower tables facing the light. Each man has three lineal feet of table room, and is provided with a gas-cock of his own, and shares a water spigot, with a small brass basin below it, with the next

FIG. 2.—Pathological Laboratory.

neighbor. A commodious hood at one end of the room contains the sterilizers and gas stoves for making culture media for the common use of all the students. A ventilator at the top conducts hot air, steam, and vapors from the laboratory. There is also a general work table with two sinks, gas outlets, outlets for compressed air and for vacuum pumps, for overflow water baths, etc., and upon

FIG. 3.—Pathological Laboratory.

FIG. 4.—Bacteriological Laboratory.

this table are placed burets with the proper solutions for titrating culture media, stock solutions of all kinds, and such pieces of apparatus — hydrogen generators, etc.—as must be used by the class in common. A large slate sink is placed at one side of the laboratory, and affords ample room for test tube

FIG. 5.—Private Laboratory.

cleaning and the washing of hands, there being a large shelf above this sink supporting large containers of such disinfecting solutions as formaldehyde, carbolic acid, and bichloride of mercury for general use. For the support of funnels, sauce-pans, flasks, and other apparatus, each table is provided with a socket into which a brass rod 14 inches long can be screwed when desired. This can be shared by two men in common, can be removed and kept in a drawer when not in use, and forms a much more stable support than the ordinary laboratory stand with the iron base. Blackboards and microscope lockers are conveniently placed between the windows, and the blank walls are provided with hooks for hats and coats.

The floors throughout the entire laboratories are of cement. The tables are all of oak, and have their tops ebonized by the hydrochlorate of an anilin process.

JOSEPH MCFARLAND.

Medico-Chirurgical College, Philadelphia.

### A New Method of Embedding Small Objects.

The difficulty and annoyance due to the usual methods of embedding in paraffin loose, minute objects, for example, Echinoderm eggs, are familiar experiences of all workers in cytology. As a matter of fact, no really satisfactory methods are at present employed, and most of them are at best awkward and tedious. In embedding in a paraffin-drop on a slide, or in using the watch-glass method, it is difficult to keep the objects close together, and even when this is obviated, as in Boveri's method of wrapping Echinoderm eggs in the sloughed epidermis of Amphibia and thus confining them, the process is an irksome and unsatisfactory one. The same is true

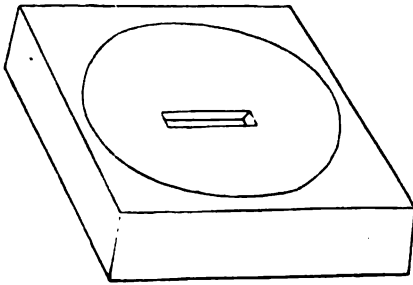


FIG. 1.

of the various methods of embedding in glass tubes, one of which is to break off the glass after cooling, leaving the objects behind in the paraffin.

To do away with all of these objections I have devised a special form of glass dish in which such objects may be embedded with the utmost ease, and which

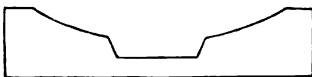


FIG. 2.



FIG. 3.

effectually prevents them from scattering. The dish is a flat, solid watch-glass, containing a shallow concavity, in the bottom of which is moulded a narrow, slot-like groove or trough. (Fig. 1.)

When the objects are ready for embedding, they are transferred to the dish, filled with melted paraffin and kept warm on the bath, by carefully dropping

them from a pipette into the groove, where, owing to the confined space, they will remain close together. The bottom of the dish is then rapidly cooled on the surface of water, and, when the paraffin is thoroughly hardened, it may be removed without difficulty. The objects are held in the portion of the paraffin which previously filled the groove, and which now projects from the surface of the block. With a little trimming the paraffin is ready for sectioning.

The dish is 40 mm. square and 9 mm. high; the diameter of the concavity is 34 mm. and its greatest depth  $4\frac{1}{2}$  mm. The groove, which is slightly beveled at the ends, is 11 mm. long on the bottom, 2 mm. wide, and 2 mm. deep. In Fig. 2 is shown a section of the glass through the long axis of the groove, and in Fig. 3 a section taken across the groove. When

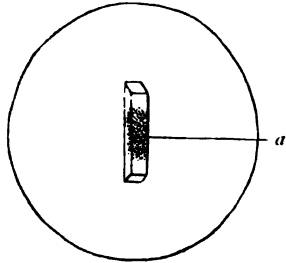


FIG. 4.

the hardened paraffin is removed from the dish it has the form seen in Fig. 4; the embedded objects are indicated at *a*. The block, when trimmed and ready for sectioning, may be cemented to a piece of wood or to the metal paraffin-holder of the Minot microtome. (Fig. 5.)

I have thoroughly tested the practical usefulness of this dish, and my experience has shown that it may be manipulated so easily and conveniently that the embedding in it of such minute objects as it is intended for becomes as simple an operation as the embedding of larger ones which may be handled individually.

The method of procedure which I have used is as follows: The objects are dehydrated in the usual manner in round-bottomed tubes, the change of liquids being made entirely by means of a pipette. After saturation with the solvent, the tube is placed on the paraffin-bath and melted paraffin added with a warm pipette. When ready for embedding, the objects are drawn into a pipette and carefully dropped into the groove of the watch-glass containing melted paraffin. A very little dilute glycerine must be previously rubbed on the inside of the dish, and also on the sides and bottom of the groove, to prevent the tendency of the paraffin to stick to the glass. If any of the objects should have failed to fall into the groove when dropped from the pipette, they may be readily pushed in with

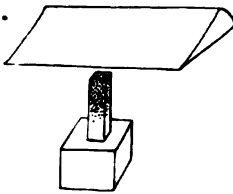


FIG. 5.

a needle or small spatula; or if the number of objects is not great, and they should scatter along the bottom of the groove, they may be pushed to one end in the same way. I also find that it is possible to dispense entirely with the round-bottomed tubes, as the whole process may be performed in the dish by keeping the objects in the groove and making the change of liquids with a pipette.

The dish is now cooled rapidly on the surface of water, and when the paraffin is thoroughly hardened, a knife edge is inserted under the paraffin *opposite one end of the groove* and the entire mass lifted up. The projection of the paraffin-mass containing the embedded objects may be sectioned with the edge of the microtome knife set either parallel to its long axis or perpendicular to it, as may be desired. In either case the front and back edges of the mass are already parallel, and need no trimming before sectioning.

In addition to its use in embedding large numbers of loose, minute objects, the dish is serviceable for the purpose of orientation. A small object lying in the groove may be rapidly oriented with a warm needle under the microscope, and placed in any desired position. It is possible then to cool the dish without disturbing the object.

GEORGE LEFEVRE.

## LABORATORY PHOTOGRAPHY.

Devoted to Methods and Apparatus for Converting an Object into an Illustration.

### FURTHER NOTES ON A NEW METHOD OF FOCUSING IN PHOTOMICROGRAPHY.

At the request of the editor, we have prepared the following notes on our method of focussing, described in full in the *Zeit. f. Wiss. Mik.* Band 18, 1901 :

The method offers special advantages for the vertical camera<sup>1</sup> and daylight illumination, as it does away with the use of the ground glass, a minus spherical lens being substituted for the purpose of focussing. These lenses can be obtained from any optician, and a series (omitting the half numbers) ranging from —1.D. to —12.D. will furnish the equipment necessary for photographing at 1200 diameters, or less, with most combinations of objective, eyepiece and bellows draw. The lens for a definite magnification depends upon the eyesight of the operator. The selection of this lens is a simple matter, and can be determined by taking one photograph.<sup>1</sup>

Our method in brief is as follows: Instead of attempting to focus on the ground glass fine details impossible to see with daylight illumination, the change of focus necessary to throw the exact image (selected for the photograph) on the ground glass, is accomplished by focussing *through* a minus spherical lens placed on top of the projection ocular. This lens is removed before the plate is exposed—the *photograph* is not taken through the lens. The use of these lenses is simply a device for compelling the eye to see the plane of the preparation that is projected on the ground glass.

For example, in photographing a centrosome at 1000 diameters (oil immer. lens, projection ocular 4, diaphragm at 0), if an operator with normal eyesight takes a sharp focus on the centrosome through the ocular, and exposes the plate *without* changing the focus, the negative will show the centrosome very much out of focus; indeed, if the centrosome is small it will not be reproduced on the negative at all. The sharpest details on the negative will be those on a plane of the preparation *lower* than the one seen through the projection ocular.

To get the correct focus for the negative, place a —6.D. spherical lens (assuming that this is the right number for the operator) on top of the projection ocular. Through this lens the eye will see the plane of the preparation that is shown in the negative just taken, and it will be necessary to raise the focus to get a sharp focus on the centrosome, *the plate requiring a higher focus than the normal eye*. Having secured an accurate focus on the centrosome *through* the —6.D. lens, remove the lens and expose the plate. The negative will now show the centrosome clearly as seen through the —6.D. lens.

<sup>1</sup> We use the Bausch & Lomb vertical camera, with the addition of a time shutter. With daylight illumination the Carbutt, backed, ortho-portrait (27) plates give very satisfactory negatives with three or four minutes exposure, for 1000 magnification, using an oil immersion lens.

<sup>2</sup> See *Zeit. f. Wiss. Mik.* Band 18, 1901.

Before exposing the plate a delay of a few minutes is necessary to see that the focus does not slip. We have been able to overcome, in a measure, the annoyance of a changing focus by discarding the spring clips that hold the slide to the stage of the microscope. Either by doing this or by using a mechanical stage, one of the factors of a changing focus is removed.

As the scale of the stage micrometer cannot be seen distinctly enough on the ground glass with daylight illumination to admit of accurate measurement, we have heretofore ascertained the magnifications of our photographs by measuring the subject with a Zeiss micrometer eyepiece (using 2 mm. objective), taking the measurement of the photograph in microns, and dividing the latter by the former. We have found that this method does not give accurate results, because the Zeiss micrometer eyepiece, as tested by the Zeiss stage micrometer, overestimates the size of the subject, thus causing the magnification of the photograph to be underrated. This can be seen by measuring the Zeiss stage micrometer by the Zeiss micrometer eyepiece. With the Zeiss 2 mm. objective and a tube

Zeiss stage micrometer ruled to 0.01 mm. spaces. In photo each space measures 10 mm., showing a magnification of 1000 diameters.

length of 153 mm. (with nosepiece), 90 $\mu$  of the stage micrometer measures with micrometer eyepiece 97 $\mu$ , and it is necessary to shorten the tube to the 140 mark to make the two scales agree.

A few photographs of the stage micrometer, taken with different combinations of lenses and bellows draw, provide an accurate register of magnifications, in convenient form for reference in selecting the lenses and draws needed for a given magnification.<sup>1</sup>

Fig. 1 is a reproduction of a photograph of a part of a Zeiss stage micrometer taken with a Zeiss 2 mm. apo. immer. lens, 140 aperture, Zeiss projection ocular 4, diaphragm at 0, tube length 153 mm. (with nosepiece) and bellows draw of 27 inches from stage of microscope to plate. The focus was taken on the faint dot in the center of line 6. This dot was placed exactly in the center of the field with the aid of the Zeiss micrometer eyepiece. We have reproduced this photo-

<sup>1</sup> With the Zeiss 2 mm. immer. lens, projection ocular 4, diaphragm at 0, we have always used a 29 inch bellows draw for about 1000 magnification. This was given in an earlier paper as 29 $\frac{3}{4}$  inches, the error arising from the measurement being taken to top of the upper collar, instead of from stage of microscope to plate, as stated. Testing this measurement with a photograph of a stage micrometer shows that 27 inches is the correct draw for a magnification of 1000.

graph to show how the lack of flatness of the field limits the area in which it is possible to get a sharp focus.

In using our method of focussing it is a great aid to determine the limits within which a sharp focus can be expected, for it is easy to strain the eye to see details beyond these limits; the negative in this case giving disappointing results.

We have not found it necessary to use color screens or the ray filter, as most of our work is done with preparations stained in iron hæmatoxylin. If preparations with this stain are very much decolorized, a short after stain of dilute Bismarck brown will give enough yellow tone to the preparation to affect the sensitive photographic plate and to insure a brilliant negative, although the color should be so delicate as to be scarcely perceptible to the eye through the microscope.

Woods Hole, Mass.

KATHARINE FOOT AND E. C. STROBELI.

## METHODS IN PLANT PHYSIOLOGY.

### VI.

#### VIII. CARBON ASSIMILATION.

1. **The Effect of Light upon Food Formation.** Select three sets of five kernels of corn (*Zea Mais*) so that each set has exactly the same weight. One set of kernels is to be crushed in a mortar, and dried in an oven at 100°C. until they cease to lose weight upon further heating; the final dry weight is recorded for future comparisons. The other two sets are planted in separate crocks of damp sawdust, and when the plumules have obtained a length of 2 cm. to 5 cm. the seedlings are to be removed, carefully washed in distilled water, and placed in a water culture containing a full nutrient solution as described on p. 2004 of this journal. The two jars are to stand side by side in the plant house, one in bright light, the other deprived of all light.

At the end of three or four weeks each group of seedlings is to be removed separately, no part being lost, washed carefully in distilled water, all adhering particles being removed, and the seedlings cut into small fragments with the scissors. Place the fragments of each group in a small beaker, and determine their dry weight as the weight of the kernels was previously determined. Compare the weight of the plants with that of the ungerminated grains. If the experiment has been successfully conducted there will be a decided gain in the case of the illuminated plants, while the unilluminated plants will show only a small gain, or possibly a loss. The difference in the two will serve to indicate the amount of food formed by photosynthesis.

2. **Effect of Light on Starch Formation.** Cover some leaves of the Nasturtium (*Tropæolum majus*), or other suitable plant, with tin foil, allowing the covering to remain for two days; or a plant may be placed in the dark for two days. Leaves that have been illuminated, and others that have been in darkness, are removed from the plant in the afternoon, are killed by immersion in boiling water, and placed in boiling 80 per cent. alcohol until the chlorophyll has been extracted. The leaves are then to be placed on a white plate and covered with

iodine solution. The presence of starch in the illuminated leaves, and its absence in the shaded ones, shows the necessity of light for starch formation.

3. **Local Effect of Light on Carbon Assimilation.** Partially cover leaves of *Tropæum*, tobacco (*Nicotiana tabacum*), or sunflower (*Helianthus*), with thin disks of cork. These disks are to be placed opposite one another on either side of the leaf, and are to be secured with weak wire clips. Place the leaves so that they will receive perpendicular rays of light for much of the day. The experiment will continue twenty-four to thirty-six hours, and be ended between four and six in the afternoon. The final treatment will be the same as in the preceding experiment.

4. **Relation of Variegated Leaves to the Assimilation of Carbon.** In the late afternoon of a bright day, gather some variegated leaves which have received strong light for most of the day. Make a sketch of the color pattern of each leaf, then kill and apply the iodine test as in the preceding work. Note that the starch pattern coincides with the chlorophyll-bearing surface of the leaf.

5. **Translocation of Starch.** Late in the afternoon of a bright day, gather some leaves from a *Tropæum*, or other suitable plant that has stood in strong sunlight. At the same time cover the plant so that no light can reach it until other leaves are plucked early the next morning. The leaves that are gathered in the evening are to be killed immediately by immersion in boiling water. They may then be put into alcohol for preservation. The leaves taken in the morning are to be killed immediately, and both sets tested for the presence of starch. The results are to be explained by the fact that the starch is transformed into sugars by the action of enzymes and translocated to other parts of the plant during the night.

6. **Formation of Starch in the Absence of Light.** Place some plants of *Elodea canadensis* in tap-water and allow to stand in the dark until the iodine test shows that the starch has all been consumed. Then select two healthy stems, determine their length, and count the leaves on each; place one stem in a large flask of a 2 per cent. solution of cane sugar, the other in a similar flask of clean tap-water. The preparations should be watched to prevent the growth of fungi, and observations made after three or four days for growth and the presence of starch. In the absence of light the plant is able to manufacture starch from any available carbohydrates.

7. **Effect of Light-waves of Different Lengths on Carbon Assimilation.** Fill a double-walled bell-jar with a saturated solution of potassium dichromate, fill a similar bell-jar with a weak solution of ammoniacal copper sulphate; under each bell-jar place a small plant of *Tropæum*, and allow them to stand in strongly diffused light. At the end of forty-eight to seventy-two hours detach some leaves from each plant and determine the relative amount of starch present by the iodine test. It will be seen that the long waves of the red end of the spectrum are more efficient than the short ones of the blue.

University of Michigan.

HOWARD S. REED.

## The Bacterial Flora of Freshly Drawn Milk.

### II.

Comment has already been made upon the fact that by far the largest number of species determined in all the samples tested were lactic acid species, and that other species, although more or less constantly present, were not invariably so, and never in very large quantities. This is a most important practical consideration, for it means that although by the most scrupulous care it may not be possible to procure milk free from germ life, because of those that are present in the udder of the cow, yet the species that gain access to the milk through this source are for the most part beneficial ones. In bulletin No. 21, March, 1900, of Storrs Agr. Expt. Sta., Conn speaks of a method now widely adopted in American dairies for procuring what is known as a "natural starter." The method consists in drawing milk just as has been done in all the examinations made for this work into sterilized flasks and using cultures from these as starters. Conn says, "There can be no question that the use of natural starters thus made has been a very decided advantage to the buttermaker" for the reason that "the bacteria which are within the cleanly cow's teats and thence get into the milk, are most commonly of the desired character." There is no doubt some uncertainty about this method, but so far as all examinations conducted by us are concerned, the cultures so obtained would be good ones, being largely composed of lactic acid species.

While the large percentage of lactic acid species present is the paramount characteristic of the bacterial flora of freshly drawn milk, yet there are other peculiarities of considerable, if not equal, importance.

By reviewing the description of the species determined, it will be noted in every case that, although each species would grow at room temperatures, yet the optimum temperature was in the neighborhood of 37°. This fact was well demonstrated in comparing gelatine plates made from the general milk supply with those made from the aseptically drawn milk. These plates cannot be kept at a temperature higher than 22°, and it was most marked that when plates from the former were quite covered with bacterial growth, those from the latter were still clear. On the other hand, when agar plates were used and kept at a temperature of 37°, the order of growth was slightly reversed. This explains facts that were noted in reference to the keeping quality of the aseptically drawn milk as compared with that of the general milk supply; for when kept at room temperatures the former remained good considerably longer than the latter; whereas, when kept at 37°, both became curdled in less than 24 hours.

Another marked characteristic of all the species was that they were facultative anærobes. This anærobic faculty was especially marked in the species, Nos. I, II, and III, that were found to be so uniformly present in all the milk tested. This is just what one would naturally expect, for the conditions in the udder must be largely anærobic conditions. The presence of anærobic conditions and a high temperature are a most wise provision, favoring as they do, in a marked manner, the multiplication of lactic acid species to the exclusion of many other

species. In virtue of these conditions and possibly other undetermined conditions, the udder, so to speak, exerts a selective action upon the bacteria which may be temporarily present in it. In this, they are of course aided by the mechanical expulsion of bacteria in the process of milking.

In order to throw a little light upon this problem, experiments were conducted in inoculating udders with well-marked but harmless bacteria which could be easily recognized by their cultural characteristics. *Bacillus prodigiosus* and *B. exiguum* (IX), both of which are marked by their pigment production, were the ones experimented with. Cultures of these were smeared upon the ends of the teats, so that the bacteria might work their way up into the udder, just as any other germ might which comes in contact with the ducts of the teat. In the case of *B. prodigiosus* about 20,000 per c. c. were present in the fore milk at the first milking 8 hours after inoculation. By the third milking, only a few were present, and after that it disappeared completely. The experiment was repeated with *B. exiguum* with similar results, although a smaller number, 240 per c. c., were present in the first milking, and by the fourth milking it had disappeared. No doubt the small number was due to the fact that this germ grows much more slowly than *B. prodigiosus* as will be seen by reference to the description (see IX).

In view of Ward's discovery of *B. fluorescens liquefaciens* in the udders of certain cows, it seemed advisable to attempt to colonize this germ in the udder, and a bouillon culture was smeared upon the ends of the teats of a cow in the manner already described. This bacillus was recovered in the foremilk six hours after the teats were smeared, but was not found in the fore milk of the second and third milkings.

It does not seem probable that an ærobie bacterium of this character is able to live and compete with facultative anærobie bacilli. Further, the optimum temperature for the fluorescing bacterium is not 37°.

Possibly, by continuous experimentation, we might have finally discovered a species which would persist in the udder, but, at the same time, the bacteria chosen have evidently fared much the same as other more or less injurious forms which may occasionally find temporary lodgment in the udder. Exception, however, may occur, as we have the gas and taint producing bacillus located by Ward and Moore in the udders of the cows of a particular herd.

Another fact that may have a bearing on this problem is that normal healthy organs, taken from the body immediately after death, may contain bacteria which are capable of development. Thus, Ford (XX) has shown that 80 per cent. of healthy organs, removed from killed guinea pigs, rabbits, dogs and cats, contained living bacteria. No udder tissue was examined by him, and in order to ascertain if bacteria existed in the udder of healthy animals, a few experiments were made along these lines, but they are open to criticism, because it is impossible to say with any certainty that the bacteria found came from the animal's glands or blood, or from infection through the teat. However, by selection of cows which had been dry for several weeks before slaughter, the latter objection is to some extent overcome. The liver was examined at the same time, and its bacterial content, if any, noted.

Ontario Agricultural College.

F. C. HARRISON,  
M. CUMMING.

## The Technique of Biological Projection and Anesthesia of Animals.

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### IX. THE ANESTHESIA OF ANIMALS.—Continued.

To produce a state of hypnosis, or complete anesthesia, in animals by the use of chloretone, either one of four methods of administration may be used, or two of them may be combined, as each case may demand.

1. Place the animals in water sufficient to cover them and add chloretone stock solution, i. e., one per cent. solution in tap water, slowly drop by drop, or in larger quantities, until the desired state is attained.

2. Place the animals in a dilute solution of definite strength, or in the full strength stock solution. Use tap water, or water in which the animals naturally live, for making dilute solutions from the stock solution.

3. Inject stock solution into the animal's stomach by means of a pipette or syringe.

4. Feed the animal with a proper dose of the chloretone crystals in capsules or sugar-coated tablets.

PROTOZOA: *Amœba*.—Although these animals do not usually move with such rapidity as to require anesthesia for their careful study in the live state, the interesting phenomena which they present under the influence of chloretone justify a description of the method.

*Amœbæ* are easily cultivated in the laboratory in shallow, flat-bottomed, loosely covered vessels, such as large crystallizing or culture dishes. If the *amœba* stock is obtained from the surface of the mud in a pond or ditch, care should be taken not to transfer enough mud to completely cover the bottom of the dish. Areas free from the coarser sediment should appear after it has settled. Let such a culture stand quietly for a few days, not in direct sunlight and free from snails, which probably destroy the *amœbæ*. When ready for study, take a clean watch-glass and a clean rubber bulb pipette having a good point of medium or small bore, and scratch the clear areas vigorously with the point of the pipette and *at the same time* allow the pipette to fill with water containing fine mud, microscopic plants, and *amœbæ*. Transfer this water and its floating contents *at once* to the watch-glass. The *amœbæ* adhere to the surface of the glass, therefore vigorous scratching is necessary to loosen them, and they quickly settle upon and adhere to the inside of the pipette, if not transferred immediately to the watch-glass.

Place the watch-glass under a one-half or three-quarter inch objective, and focus carefully on the surface of the glass at the bottom of the water. In a few minutes the *amœbæ* will be found adherent to and flowing over the surface of the glass, provided that the glass and water are at ordinary room temperature, or a few degrees above. To get rid of the dirt, other organisms, and nearly all of the water, pour it off by a quick tilting of the watch-glass and the *amœbæ* will be found, on again examining with the microscope, to be moving about in

the thin film of water adhering to the glass. When their pseudopodia are seen to be well extended, add quickly a pipetteful of one per cent. chloretone solution. In fifteen seconds, or less, the amœbæ will be quiet, the pseudopodia will be extended, but they will not adhere to the surface of the glass. Amœbæ treated thus for fifteen minutes were revived in two or three minutes by the addition of a pipetteful of water. A small portion of them was killed by the treatment, indicating that fifteen minutes in a one per cent. solution of chloretone is about the limit of endurance of the species tested. This rapid recovery of the animals from the effect of the maximum non-fatal dose of chloretone on the restoration of partially normal conditions is characteristic of its action on nearly all species of animals thus far tested.

*Infusoria.*—Place specimens of stentor in a watch-glass or large hollow-ground cell containing about ten drops of water. Add one drop of one per cent. chloretone solution and observe the effect, which will be evident in one or two minutes. Add more chloretone solution drop by drop until the desired effect is produced on the general body of the cell, the larger cilia and the smaller cilia. If too much chloretone is added at one time, the animals contract strongly and do not quickly expand. Specimens were under observation for nearly three hours, during which time twelve drops of one per cent. chloretone solution were added to the original ten drops of water. Evaporation of the water and volatilization of the chloretone were continually changing the liquid, but the animals were under control and exhibited as much general and ciliary activity at any time as suited the purpose of the study. Ciliary activity is almost normal, even when the effect of the anesthetic on the body of the cell is so marked that the animal fails to respond to the stimulus of a needle prick.

The great variation in the susceptibility of different species to chloretone is well shown by the following statement of the conditions observed in an experiment with stentor about two hours after the first drop of chloretone had been added, and after nine drops of one per cent. chloretone had been added to the original ten drops of water which contained stentors, amœbæ, and a ciliated infusorian of undetermined species. All of these had been subjected to exactly the same treatment as they were in the same watch-glass; but the stentor was gradually expanding while its cilia were inactive and vacuole pulsating, amœbæ were active, and the infusorian was in the first stage of fission and completed the process in five and one-third minutes. This differential action of the chloretone is especially noticed between species, not between different normal individuals of the same species. Hence, when the strength of solution has been determined for a species, practically constant results will be obtained in subsequent application of the same strength solution to that species. In working out the strength of solution best suited to produce the desired effects by the first method, it is best to place the animals in a measured quantity of water, the unit of measure being a drop, a cubic centimeter, or larger unit according to the size of the animals, and a careful record should be made of the amount and strength of the chloretone solution added. Such a record is a safe guide for use in applying the second method to the same species.

The directions given above apply to animals of all types. In subsequent issues exact formulæ for the treatment of various types of animals will be given, but formulæ may be determined easily by any workers who can not wait for the full details to be published.

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## ELEMENTARY MEDICAL MICRO-TECHNIQUE.

For Physicians and Others Interested in the Microscope.

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## XI.

## HAEMAGLOBIN.

To estimate the Hæmaglobin, clean the finger or lobe of the ear with soap and water, alcohol and ether, dry and puncture with the blood lancet. Fill

the graduated pipette of a Gower's Hæmaglobinometer to the 20 cmm. mark. Put in the graduated tube a little distilled water and discharge the blood from the pipette in it, wash out the pipette with a little distilled water, discharging this into the mixture in the tube. Add water drop by drop, shaking the tube each time until the color matches the color in the gelatine tube. The colors match in daylight. Holding a piece of white paper behind the tubes assists in matching the colors. The percentage

Gower's Hæmaglobinometer.

of hæmaglobin is read direct from the height of the fluid in the graduated tube. Normal blood seldom reads higher than 95 to 98 per cent.

Fleischl's Hæmometer is a more accurate instrument for estimating the Hæmaglobin. Draw the blood as described. Apply one of the small metal handled pipettes to the blood drop so that it is filled. Empty, and wash out the blood in one side of the comparison cell which has previously been half filled with distilled water. When removing the tube wash it with a few drops of distilled water, which should go in with the other water. Mix the blood and water thoroughly with the metal handle of the

Fleischl's Hæmometer.

pipette. Now fill both compartments level full with distilled water, adjust the wedge underneath, and set the comparison cell on the stage of the instrument so that the side filled with water only may be above the colored wedge of the instrument. Examine at once in a darkened room with artificial light (candle or gas) as the colors do not match by daylight. By means of the milled head the wedge may be adjusted until the colors of the two compartments match. The percentage of hæmaglobin is read from the graduated scale—95 to 98 per cent. hæmaglobin representing normal blood. It is seldom that blood is found that reaches 100 per cent.

## MICROSCOPICAL EXAMINATION OF BLOOD.

For making micro-preparations of blood, clean covers and slips are necessary for the best results. Wash the covers in soap and water, alcohol, and a mixture of alcohol and ether. Clean the point selected for the lancet with soap and water, alcohol and ether. It must be perfectly dry. Wipe away the first drop of blood, and touch the end of a clean slip to the next drop and rapidly apply it to a cover held in a Cornet forceps, drawing the slip over the cover so as to make a thin spread on the cover. This should be done rapidly, i. e., within two or three seconds. The cover must not be touched with the finger, as the moisture would crenate the cells. Dry in the air rapidly and drop it into equal parts of 95 per cent. alcohol and ether for two hours, after which it may be stained.

The heat method of fixation gives better results except in some few cases when special stains are to be used. In all ordinary cases it should be used. Spread the blood on a clean cover-glass as directed above, and when dry put on a blood table (Huber's) which should be heated at one end by an alcohol lamp or Bunsen burner. Test the heat of the table by a small drop of water. Put the covers, blood side down, on the table at the point just where the drop of water boils and turns into steam. Fix for fifteen to thirty minutes. Stain with any of the following stains:

**EHRlich's TRIACID STAIN.**—This stain is so difficult to make that it should be purchased of some reliable dealer. Orange G., acid fuchsin, and methyl violet are the dyes which compose it. Apply all of the stain that will remain on the cover, which should be held in a Cornet forceps. Stain for fifteen minutes, wash in water, dry between pieces of filter paper, and mount, blood side down, on a clean slip, in a drop of balsam. The nuclei of the leucocytes will be stained greenish; the eosinophile granules, copper color; neutrophile granules, violet. The nuclei of basophile leucocytes will be pale green, while the protoplasm will be unstained. Red corpuscles will be stained orange. If they take a reddish tint it will be due to anæmic degeneration.

**HÆMATOXYLIN AND EOSIN STAIN.**—Stain two minutes in 1 per cent. yellowish eosin in 70 per cent. alcohol, wash thoroughly and apply Delafield's hæmatoxylin for three minutes, wash again thoroughly, dry, and mount in balsam. The red cells will be stained red, the nuclei of white corpuscles will be blue.

**JENNER'S STAIN** is a very useful one. It is prepared as follows:

SOL. I	{	Yellowish Eosin,	-	-	-	1.2 grams.
		Distilled water,	-	-	-	98.8 grams.
		Mix.				
SOL. II	{	Methylene Blue,	-	-	-	1 gram.
		Distilled Water,	-	-	-	99 grams.
		Mix.				

These solutions should be thoroughly mixed in an open vessel and set aside for twenty-four hours. Filter and dry the sediment. Powder this, wash it with water, and again filter and dry. The powder so obtained should be used in  $\frac{1}{2}$  of 1 per cent. solution in pure methyl alcohol. Add 10 per cent. glycerine, and it is ready for use. The stain is applied to the blood preparation, which requires no fixation. Stain for from one to three minutes, wash in water till the film is pinkish in color, dry and mount in balsam. Red cells are stained terra cotta color, leucocytes blue, neutrophile granules bright red, and basophile granules violet. Malarial organisms and bacteria are stained blue.

The stain is a very important one, easy to use and rapid, as no fixation of the blood is required. For success the covers must be thoroughly clean. Wash them in soap and water, ammonia and water, ether, and lastly absolute alcohol. Dry without touching the surface with the fingers.

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Harvey Medical College.

## A Review of the Methods of Staining Blood.

### III.

#### B. *Acid Stains.*

1. **Azoblue.**—Griesbach (1886) used Azoblue for staining the blood of fish and tritons. The acidophile granules are stained red.

2. **Congo Red.**—Stintzing (1889) stained blood preparations with Congo red which differentiated what he called *Congophile* granules in certain leucocytes. These were undoubtedly acidophile granules.

3. **Eosin.**—This is one of the most important acid stains for the blood. The acidophile granules of the blood were first called eosinophile by Ehrlich because of their affinity for this stain. It enters into the composition of many staining solutions used in the study of the blood, including the neutral eosin-methylen blue staining solutions. Used alone it stains all acidophile elements of the blood eosin red. All of the eosin solutions require well fixed preparations.

*Bizzozero* (1884) stained heated preparations of the blood one-quarter hour in a dilute watery solution of eosin.

*Ehrlich* recommends a strong solution of eosin in glycerine for staining "eosinophile" granules. Well fixed preparations should be stained at least 15 minutes.

Eosin is often combined with one or more other acid dyes for staining the blood on the supposition that not all acidophile elements are eosinophile.

4. **Eosin, Aurentia, and Indulin.**—*Ehrlich* employed three dyes dissolved in glycerin. With this mixture all hemoglobiniferous parts are stained an intense pure orange, nuclei blackish, acidophile granules red or reddish black.

*Schwarz* (1880), who worked under *Ehrlich*, used the following mixture: 1 volume of glycerin saturated with aurentia is mixed with one to two volumes of glycerin. To this mixture is added eosin and aurentia in excess. Saturation follows through long shaking.

*Huber* and *Becker* (1886) dissolved two grams of eosin, aurentia, and indulin in thirty grams of glycerin. The mixture should be thoroughly shaken before using. Stain heated preparations one-half hour to several days.

5. **Eosin, Aurentia, and Nigrosin.**—*V. Norden* (1891) gives the following formula for an eosin, aurentia, and nigrosin mixture:

Eosin (cryst.),	-	-	-	-	-	-	3.0
Aurentia,	-	-	-	-	-	-	2.0
Nigrosin (watery solution),	-	-	-	-	-	-	5.0

These are rubbed together with best, water-free glycerin in a mortar, and at the same time warmed in a sand bath to 80° or 90°C. The viscous mass is then placed in a wide-necked flask, which is stopped with a cork to keep out the steam, and heated for several days in a water bath at 60°C., being thoroughly shaken from time to time. Heated preparations are to be stained 24 to 36 hours, washed, dried, and mounted.

6. **Eosin and Nigrosin.**—*Ehrlich* and *E. Newman* used an eosin and nigrosin mixture for the study of nucleated red corpuscles.

7. **Orange G.**—Ehrlich recommends a saturated watery solution of orange G. as a stain for the acidophile granules of the blood.

C. *Double Staining with Acid and Basic Stains.*

1. **Aurentia and Methylen Blue.**—Müller stained dry blood preparations that had been heated two hours at 115° to 120° C. with aurentia followed by methylen blue. A drop of a concentrated alcoholic solution of aurentia is placed on the preparation, spread, and allowed to evaporate. The preparation is treated in the same way with a second and a third drop. It is then washed in alcohol until no more stain dissolves out, and stained in a strong watery solution of methylen blue for from 5 to 10 minutes, or more, then washed in water, dried in the air and mounted. Red corpuscles are stained yellow, nuclei of nucleated red corpuscles green, nuclei of resting mono-nuclear leucocytes pure blue.

*Bizzozero* (1890) used aurentia and methylen blue for recognizing hemoglobin in the early development of red corpuscles in birds.

*Castellino* (1892) employed aurentia and methylen blue for the study of the degeneration of the red corpuscles.

2. **Eosin and Hæmatoxylin.**—Ehrlich's eosin and hæmatoxylin mixture is as follows:

Eosin (cryst.),	-	-	-	-	-	-	0.5.
Hæmatoxylin,	-	-	-	-	-	-	2.0
Absolute alcohol	-	-	-	-	-	-	100.0
Distilled water,	-	-	-	-	-	-	100.0
Glycerin,	-	-	-	-	-	-	100.0
Glacial acetic acid,	-	-	-	-	-	-	10.0
Alum in excess.							

This mixture must ripen some weeks before use.

Preparations should be fixed in absolute alcohol or by short heating, and stained several hours. The staining solution should be very carefully washed off. Hæmoglobin and acidophile granules stain red, nuclei hæmatoxylin color. Orange G may be substituted for eosin in this mixture.

3. **Eosin and "Methylanilingrün."**—More and Stowell (1884) stained blood preparations with eosin (1: 50 water and 50 alcohol), dried, stained with "methylanilingrün" (1: 100 water), dried, and mounted.

Massachusetts State Board of Health.

ERNEST L. WALKER.

## LABORATORY OUTLINES.

For the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution.

XX. *Hydrodictyon reticulatum* (L.) Lag. Water-net. Order, Hydrodictyales. Family, Hydrodictyaceæ.

The water-net forms a large body, which is common in summer and autumn in ponds and canals. It may often be collected in great quantities along the

grassy banks of ponds in city parks. The body of the alga is made up of a very great colony of cylindrical cœnocytes arranged in the form of a sack-like net.

1. Examine a large plant and describe the naked-eye characters.
2. Draw a small portion of a young net under low power, showing how the meshes are formed by the joining of a number of cœnocytes. Describe.
3. Under high power draw a single cœnocyte, showing the chloroplast and numerous pyrenoids.
4. Vegetative propagation. Study and draw a large cœnocyte of an old net in which the cells are developing a daughter net.

**XXI. *Cladophora* Sp. Family, Cladophoraceæ.**

Species of *Cladophora* are commonly found in flowing water. They appear as large, dark green, extensively branched tufts attached to rocks and pieces of wood. These algæ are also abundant along the shores of lakes, where they may often be found attached to objects which are exposed to the action of waves.

1. Describe the naked eye characters; size, mode of growth, color, habitat, etc. Note the differentiation of base and apex.
2. Mount a small branch of the thallus in water and study under low power.
3. Under high power study the stages showing the development of a branch. Make four drawings showing four general stages: (1) a small bulging out of the cell wall on one side below a septum; (2) a short branch with the protoplasm still connected with the parent cœnocyte; (3) the branch cut off from the parent cœnocyte by a septum; (4) a branch divided by a transverse septum into two cœnocytes.
4. Draw a single cœnocyte or division between two cross walls, showing the irregular chloroplasts and the pyrenoids. Notice the large central vacuole. Apply salt solution and note the effect. Numerous nuclei are present, but these can probably not be distinguished from the pyrenoids without special staining. Draw. It is a cœnocytic plant with numerous transverse walls, but the walls do not represent cell divisions. How and where do the branches always originate?
5. Draw and describe an empty cœnocyte or zoösporangium from which zoöspores have escaped. Where is the opening (ostiole) always formed?
6. At certain times zoöspores may be seen forming and escaping into the water. This frequently occurs in material which has been kept in water in a warm room. Draw a cœnocyte in which zoöspores are developing.
7. Draw a cœnocyte in which the zoöspores are fully formed. Look for zoöspores in the act of escaping through the ostiole.
8. Study and draw free-swimming zoöspores, showing the chloroplast and red eyespot. To make the two flagella visible treat with iodine solution by placing a drop on the slide beside the cover glass and letting it mix slowly with the water of the mount.
9. Draw a zoöspore which has begun to develop into a new filament.
10. The zoöspores (planogametes) of some species are said to conjugate. Look for such a process.

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THE present number closes the fifth consecutive year of publication of the JOURNAL OF APPLIED MICROSCOPY AND LABORATORY METHODS. Since its beginning, our single purpose has been to make it a journal of work for workers by workers, and its function has not only been the publication of original work within the field of methods and apparatus, but also the publication of compilations of methods on specific subjects, making them readably accessible. The result has been, in the five volumes already issued, a very large mass of work, methods, and formulæ, brought together in convenient form for daily reference, and the fact that very large numbers of complete sets have, within the past two years, been ordered by libraries and laboratories, encourages us in our policy of broadening the scope of the JOURNAL still more, as we have progressively done each year, until, when we have succeeded in obtaining the support of workers in biological sciences very generally, we hope to make the JOURNAL a hand-book of laboratory work, not only confined to the microscope, but including field methods employed by the student of animals and plants; the how and why of the teaching museum and practical reference work for the laboratory lanternist, in fact, to cover that ground relating to methods and apparatus of a biological character which is not covered by any other publication in this country.

The opposition which we encountered at the beginning of our publication, through the belief of many that the JOURNAL was not an independent publication, and that its reading pages would be hampered through relation with its publishers, has entirely disappeared, and our five years of steadfast adherence to our original purpose of publishing a purely scientific and useful work has gradually brought us the approval of so many of our leading workers, that we feel encouraged to ask, during the coming year, a still more general publication of papers within our field in the JOURNAL, in order that it may become what we have hoped it would, a truly representative American publication.

We have arranged for several very important series of articles for the coming year, and many very interesting and useful papers. The JOURNAL will be published hereafter entirely on fine coated book stock and no effort will be spared to use as many illustrations as can profitably be employed to illustrate the text.

## CURRENT BOTANICAL LITERATURE.

CHARLES J. CHAMBERLAIN, University of Chicago.

Books for Review and Separates of Papers on Botanical Subjects should be Sent to Charles J. Chamberlain, University of Chicago, Chicago, Ill.

Dixon, H. H. Sectioning without Imbedding. Notes from the Botanical School of Trinity College, Dublin. No. 5, August, 1902.

Attention is called to the fact that paraffin sections are often unsatisfactory where complexes of lignified and

delicate walls are to be prepared. Free hand sections, while often good over small areas, are seldom good as a whole, especially if the object be large. Professor Dixon recommends the microtome for cutting vegetable material which has not been embedded. Soft material, like leaves and herbaceous stems, should be left in alcohol to harden for at least a few days. Harder tissues, after a few days immersion in alcohol, should be placed in a mixture of alcohol and glycerine, adding more glycerine the harder the specimen. The material is clamped in the microtome, and kept wet with glycerine and alcohol during the cutting. The knife should be placed obliquely as in cutting celloidin sections. For transverse sections of stems, roots, etc., the object is easily fastened, but it is rather difficult to make longitudinal sections of small, cylindrical organs.

Sections as thin as  $10\ \mu$  may be cut in this manner. Wood of *Pinus sylvestris* (White Pine), after treatment with equal parts of alcohol and glycerine, gives excellent sections by this method. If sections of the entire stem, showing both wood and bast are desired, about two parts of alcohol to one of glycerine is a better mixture.

It is hard to make complete sections of monocotyl stems, like *Zea Mais*, on account of the mixture of very soft and very hard tissue. Material, after treatment with 90 per cent. alcohol and "a trace of glycerine," yielded excellent sections  $10\text{--}12\ \mu$  thick.

Although this method of section cutting is subject to very definite limitations, it is extremely useful where it can be applied.

C. J. C.

NOTE.—In the reviewer's laboratory, complete transverse sections of the stem of *Zamia* have been cut without embedding. Some of the sections, two and one-half inches in diameter, are not more than  $20\ \mu$  in thickness. The specimens were fastened into the microtome by means of an improvised clamp, and the knife was placed obliquely as for celloidin sectioning.

Murbeck, Sv. Ueber Anomalien im Baue des Nucellus und des Embryo-sackes bei parthenogenetischen Arten der Gattung *Alchemilla*. Lunds Universitets Arsskrift. 38: 1-11, pl. 1, 1901.

Since parthenogenesis in the flowering plants has been proven in only three genera—*Antennaria* by Juel, *Alchemilla* by Murbeck, and in *Thalictrum* by

Overton—it is interesting to note any accessory peculiarities. The most important observation is that the number of chromosomes remains unchanged throughout the entire life history, not showing any reduced number in the gametophyte stage. The behavior of the antipodal nuclei and synergids is also

peculiar in *Alchemilla*, some or all of these five nuclei retaining the power of motion so that they behave like polar nuclei. Consequently it is not uncommon to find three or four nuclei at the middle of the sac where one expects to find the two polar nuclei. In such cases the extra nuclei clearly belong to the synergids or antipodals, these regions showing a corresponding lack. Associated with parthenogenesis in *Alchemilla* is the phenomenon of polyembryony, the extra embryos coming from the synergids or from the cells of the nucellus.

C. J. C.

## CYTOLOGY, EMBRYOLOGY, AND MICROSCOPICAL METHODS.

AGNES M. CLAYPOLE, Throop Polytechnic Institute.

Separates of Papers and Books on Animal Biology should be sent for Review to Agnes M. Claypole,  
55 S. Marengo Avenue, Pasadena, Cal.

Holmgren, Niles. Ueber das Verhalten des Chitins und Epithels zu den unterliegenden Gewebearten bei Insecten. Anat. Anz. 20: 480-488, 8 figs., 1902.

Ueber die morphologische Bedeutung des Chitins bei den Insecten. Anat. Anz. 21: 373-378, 5 figs., 1902.

The materials used were the oviduct, spermduct and sheath of *Sarcophagus* and *Musca*, and the thoracic musculature of *Chironomus* larvæ. Tissue was killed in Perenyi's, Von Rath's, Flemming's and Carnoy's mixtures and in

sublimate (concentrated solution in normal salt). Perenyi, Carnoy and sublimate gave the best results. Sections were cut 2-3  $\mu$  thick and stained 24 hours in 2 per cent. hematoxylin solution after 24 hours preparation in 2 per cent. iron-alum; Congo red was used as a counterstain. Three kinds of chitin formation were observed. One (vagina of *Sarcophagus carnaria*) consisted of numerous parallel lamellæ following in direction the free border of the lining epithelial cells. These cells are low cylindrical with moderately large nuclei, poor in chromatin; the cell bodies are completely filled with parallel fibrils placed at right angles to the layers of chitin. Another type is found in the sperm ducts. The thick layer of chitin is here composed of very many slender chitin columns or threads lying at right angles to the surface and placed on a layer of substance that stains deeply in iron hematoxylin. The matrix cells are low, flat, cylindrical epithelium about equal in thickness to the layer of chitin. Nuclei are moderately large and rich in chromatin. Apparently the chitin is formed by the chitinization of the free border of the cells themselves, the vertical striæ of the epithelium being preserved as chitinous columns. The median oviduct of *Musca* shows still another kind of formation; here the chitin has no visible structure and reacts but feebly to stains. The matrix cells are small and spindle-shaped, drawn out to a point toward the lumen of the duct. These points project up through the chitin layer nearly to its surface. The nuclei are moderately large and rich in chromatin; the cell-bodies are striated with the striæ pointing outwards and penetrating into the structureless chitin. In all these cases the chitin is due to the activity of epithelial cells, in the rest it orig-

to pure paraffin was reduced to a few seconds to prevent shrinkages. The author urges the value of many series for comparison to eliminate individual peculiarities, of the full study, careful delineation and measurement of embryos prior to sectioning, and of the graphic and plastic methods of reconstruction in embryological investigation. Some of the excellent drawings were outlined on blue-prints, which were washed out and then finished as desired.

C. A. K.

**Illigworth, J. F.** The Anatomy of *Leucapina crenulata*. Zööl. Jahrb., Abth. f. Anat. u. Ontog. 16: 449-491, pls. 31-33, 1902.

The killing fluid used was Van Gehuchten's alcohol-acetic-chloroform mixture in which the absolute alcohol was re-

placed by 95 per cent. This was injected through the buccal sinus. This fluid whitens the muscular tissues, while the nerves retain their yellowish tint. For the finer nerves 5 to 10 per cent. nitric acid was similarly injected and the animals were then macerated for a month or more in weak nitric acid in the light. This gives a yellowish tint to the muscles, leaving the nerves a lighter hue. For nerve dissecting a glass tube was used drawn to a capillary point and bevelled. This is attached to a hydrant by a rubber tube. The sharpened end is used as a dissecting needle and a stream of water passing through it serves to remove the debris of dissection.

C. A. K.

**Moroff, Theodor.** Ueber die Entwicklung der Kiemen bei Knochenfischen. Arch. f. mik. Anat. und Entwickl., 60: 428-459, Taf. 21, 22, 1902.

Embryos of the trout (*Trutta fario*) killed at intervals of one to four days were used for this investigation. Picro-acetic, chrom-acetic, and sublimate

were used for fixing, the last giving the best results. Thin sections were double stained in hæmatoxylin-eosin or picro-acid fuchsin. Sublimate material stained with the former was well differentiated, the cartilage staining a dark violet, the other tissues a violet-blue, and the blood corpuscles red. The chrom-acetic material was somewhat better preserved, but stained with difficulty.

C. A. K.

**Bigelow, M. A.** The Early Development of *Lepas*, a Study of Cell-lineage and Germ-layers. Bull. Mus. Comp. Zööl., Harv. 40: 61-144, pls. 12, 1902.

Eggs of *L. anatifera* in all of the early stages of development were secured from adults on drifting timber at Wood's Hole in August. Early stages

of *L. fasciolaris* were rarely found. Development from egg to nauplius takes place in the mantle chamber, the eggs being found in the cavities of the egg plates between the body and the mantle. The eggs are conveniently fixed *in situ* in the plates. Only reagents containing picric acid were satisfactory. Kleinenberg's stronger fluid and a saturated solution of picric acid in 35 per cent. alcohol gave excellent fixation. A saturated solution of picric acid in 5 per cent. acetic acid gives superior results in penetration and subsequent transparency of the eggs. Less than 5 per cent. acetic acid fails in penetration. Mercuric chloride caused opacity, but was valuable in differentiating yolk in subsequent staining. Entire eggs from the picro-acetic material were stained in Grenacher's alcoholic (35 per cent.) borax carmine, decolorized in 70 per cent. alcohol + 0.3 per cent. HCl. Clove oil and especially cassia oil gave the best results as clearing agents. Sections were stained in diluted Delafield's hæmatoxylin and counterstained for yolk with eosin or orange G.

C. A. K.

## GENERAL PHYSIOLOGY.

RAYMOND PEARL, University of Michigan.

Books and Papers for Review should be Sent to Raymond Pearl, Zoölogical Laboratory,  
University of Michigan, Ann Arbor, Mich.

**Brinckley, W. J.** Physiology by the Laboratory Method for Secondary Schools. Chicago (Ainsworth & Co.), 1902, pp. xv and 536. Price, \$1.25.

The underlying general plan of this book is to present, professedly to the secondary school student, the essential facts and principles of human physi-

ology by means of the laboratory method of teaching, in which the student himself performs experiments and draws conclusions therefrom. The discussion of each subject in the text is prefaced by directions for a considerable number of experiments and other operations designed to illustrate that particular subject. While this general plan has much to commend it, the practical working out of it in this book is in detail faulty. The directions given for the laboratory work are very comprehensive, and involve a large number of experiments, but many of them are of very doubtful value. The general criticism which must be made against the laboratory directions is that they give the impression of not having been critically "tried out" in the laboratory and adapted to a specific course to be given to students of a specific grade. In other words, directions for experiments are given frequently, which would be, theoretically, very fine things in a laboratory course, but which it would be almost or quite impossible to put into advantageous practice in the average secondary school, because of lack of time in the first instance, and for the further reason of lack of technical skill on the part of the students. Thus, on the *second page* of the book the student is directed to "remove a small portion of the skin from the leg or soles of the rabbit. . . . Harden the specimen by placing it in a 2 per cent. solution of chromic acid for a week. Then transfer to 60 per cent. and then 80 per cent. alcohol. Make a vertical section, and stain in picro-carmin, mount section in glycerine, and observe," etc., etc. A further example of the generally uncritical character of the laboratory directions is noted in the fact that occasionally, fortunately not frequently, an experiment which comes dangerously near to absurdity is introduced. For example, on page 1, the student is directed as follows: "Place some sand and some wheat grains in a box where they will be free from moisture and sunlight. Put some grains of sand and some grains of wheat in soil which you have placed in a box of convenient size, and put it in a warm place where they will have plenty of sunlight. Keep them well watered. Examine them every day, and note any changes that take place." And again, further along in the book (pp. 334, 335), the student is asked to determine how many miles of tube all the sweat glands in the body would make if they were placed in line, end to end? Assuredly this is not physiology. The general character of the experiments in the last two-thirds of the book is, on the whole, good, and by a judicious selection of really significant and practical experiments

from the profusion of all sorts which the author gives, one could make a very good laboratory course in elementary physiology.

The text proper is, as is usually the case in works of this sort, in large part a compilation from various standard authorities. The first three chapters, which are entitled respectively "Introduction," "Microscopic Structure of the Human Body," and "Anatomical Elements," are distinctly far below the remainder of the book in character. These introductory chapters abound in very curious and surprising, and in some cases absolutely incorrect, statements. We are told, for example, on page 39, that all parts of the body other than the cell are "mere skeletal structures." On the same page, in a footnote, is given an account in brief of the development of the frog's egg, illustrated by a series of figures on page 43. Anyone familiar with the development of this egg will find this account, and especially the figures (which are original with the author), truly wonderful. The remainder of the text is very much better than these first three chapters. It contains considerably more useful information than the usual high school text. The style throughout is rather diffuse, and the accounts could have been made much clearer and more comprehensible for a young student if most of the unnecessary detail had been omitted. An appendix, devoted mostly to practical hints to the teacher, and a glossary of technical terms are included. The illustrations are numerous and, considering the price of the book, as well done as could be expected. The book forms, on the whole, an excellent, suggestive hand-book for the intelligent *teacher* of physiology, and therein appears to the reviewer to lie its chief value.

R. P.

Jennings, H. S., and Jamieson, C. Studies on Reactions to Stimuli in Unicellular Organisms. X. The Movements and Reactions of Pieces of Ciliate Infusoria. Biol. Bull. 3: 225-234, 1902.

Experiments were performed with cut pieces of the body of specimens of *Stylonichia*, *Oxytricha*, *Stentor coerules*, *Spirostomum* and *Paramecium*, for the purpose of determining how separated parts of the body would react to stimuli. "The operation of cutting the animals was done with a small knife, under the Braus-Drüner stereoscopic binocular, which has great advantages for such work. It was found best to cut the animals when resting against a clean glass surface. Most of the operations can be performed by the aid of some patience without great difficulty." The movements and reactions of pieces of the body in all the species studied were found to be essentially similar to those of the entire animals, in case the pieces were not too minute or too irregular in form. The pieces in swimming describe a spiral path, just as do the whole organisms. Pieces forming from one-fourth to one-half the entire animal, whether taken from the anterior or posterior end, or from the right or left side, or from the middle of the body, react to chemical and mechanical stimuli by backing and turning towards the same structurally defined side, in the way typical for the entire organism.

R. P.

Aschoff, L. Ehrlich's Seitenkettentheorie und ihre Anwendung auf die künstlichen Immunisierungsprozesse. Zusammenfassende Darstellung. Sammelreferat in Zeitschr. f. Allgem. Physiol. Bd. 1, Heft. 3 and 4, pp. (Referate) 69-248. Taf. 7, 1902.

This clear and comprehensive exposition of Ehrlich's "side-chain" theory of the action of toxins and antitoxins will be found very useful by biologists and physiologists. All the recent literature on the subject of immunity is carefully summarized, and the reviewer is to be congratulated on having succeeded so well in making this decidedly involved subject comprehensible to the general biological reader. An idea of the thoroughness of the work may be gained from the fact that the bibliography alone occupies forty-one closely printed pages.

R. P.

## NORMAL AND PATHOLOGICAL HISTOLOGY.

JOSEPH H. PRATT, Harvard University Medical School.

Books for Review and Separates of Papers on these Subjects should be Sent to Joseph H. Pratt,  
Harvard University Medical School, Boston, Mass.

**Inowye.** Ueber das Verhalten des elastischen Gewebes bei Magen Carcinom. Virchow's Archiv., Bd. 169, pp. 278-284, 1902.

Since Weigert devised a new staining method for elastic fibers, many investigators have examined the relation

of elastic tissue to different normal and pathological processes. In tumors, two views are held. By the first a new formation of elastic tissue is believed to occur, while the second claims in all cases there is a displacement and a destruction of the elastic fibers. Inowye studied twenty cases of carcinoma of the stomach to note the relation of this tissue in such cases. The specimens of seven of them had been preserved in alcohol, but the others were obtained fresh. These were subsequently studied in this condition and after they had been hardened in alcohol and formalin. The sections were first stained in borax carmine and then by Weigert's method. In order to completely decolorize the connective tissue Minervini's method of differentiation was employed. Inowye found that the elastic tissue was frequently displaced and even destroyed. It was but rarely newly formed. If new fibers were seen, they followed the arrangement and distribution of the pre-existing fibers. He was unable to find the cause for their new formation or degeneration.

W. R. S.

**Ople.** The Causes and Varieties of Chronic Interstitial Pancreatitis. Am. J. Med. Sc. 123: 845-868, 1902.

In this valuable article the causes and varieties of chronic interstitial pancreatitis are discussed at length. The

clinical records and the autopsy protocols at the Johns Hopkins Hospital were made use of, and twenty-nine cases were found. Seventeen occurred in males, and over two-thirds in persons between the ages of forty and sixty years. In ten of the cases, chronic inflammation followed partial or complete obstruction of the pancreatic ducts, while one case was associated with hæmochromatosis. He distinguishes two types of chronic inflammation: (a) interlobular; (b) interacinar. In twenty-one cases the inflammation was interlobular, in eight interacinar. The clinical and pathological report is then given separately of each of his cases. He ends his interesting paper with the following conclusions, which I give in full:

1. Chronic interstitial pancreatitis is slightly more frequent in males than females. Two-thirds of the total number of cases occur between the ages of forty and sixty years.

2. The most frequent cause of chronic pancreatitis is obstruction of the duct of Wirsung, due to pancreatic calculi, to biliary calculi in the terminal part of the common bile duct, or to carcinoma invading the head or body of the gland. Duct obstruction may be followed by the invasion of bacteria, which take part in the production of the resulting lesion.

3. Ascending infection of the unobstructed duct of Wirsung may follow an acute lesion of the duodenum or of the bile passages, and may cause chronic inflammation. In cases which have given a history of long, persistent vomiting, chronic diffuse pancreatitis may be found at autopsy, and is probably the result of an ascending infection of the gland.

4. General or local tuberculosis is occasionally accompanied by chronic diffuse pancreatitis, affecting chiefly the interstitial tissue of the gland.

5. Chronic interstitial pancreatitis is not infrequently dependent upon the same etiological factors, notably alcohol, which produce cirrhosis of the liver, and in about one-fourth of the cases the two lesions are associated.

6. Following duct obstruction and ascending infection the lesion affects principally the interlobular tissue, only secondarily invading the lobular tissue and sparing the islands of Langerhans. Diabetes results only when the lesion is far advanced.

7. Accompanying the so-called atrophic or Lænnec's cirrhosis of the liver, the pancreas is at times the seat of a diffuse chronic inflammation characterized by diffuse proliferation of the interacinar tissue, which invades the islands of Langerhans. A similar lesion accompanies hyaline degeneration of the islands of Langerhans, and the condition known as hæmochromatosis.

8. Interacinar pancreatitis is usually accompanied by diabetes mellitus. When diabetes is absent, the lesion is of such slight intensity that the islands of Langerhans are little implicated.

W. R. S.

**Steele.** A case of Chronic Interstitial Pancreatitis, with Involvement of the Islands of Langerhans in a Diabetic. *Am. J. Med. Sc.* 124: 71-76, 1902.

Thirty-five cases of diabetes have been reported which showed at autopsy an atrophy of the pancreas which involved the islands of Langerhans. These cases are divided into four groups, viz.: 1. Those where the atrophy is confined to, or greater in the islands. 2. Those where hyaline degeneration of the islands exists. 3. Those where chronic interstitial pancreatitis is found with secondary and late involvement of the islands. 4. Those where necrotic destruction of the organ results, involving the islands.

Steele's case belongs to the third group. The pancreas at the autopsy presented evidences of the increase in fibrous tissue. The sclerosis was interlobular as well as interacinar in type. In some places the connective tissue had caused the entire destruction of the pancreatic cells by pressure atrophy, and had taken their places by the formation of broad bands of fibrous tissue in which were embedded the islands of Langerhans and remnants of the much degenerated acini. The parenchyma cells showed degenerative properties. The islands were diminished in size and number, and in places the cells seemed considerably compressed and somewhat degenerated. Occasionally an invasion of these islands by connective tissue was made out, which seemed to follow the capillaries. No hyaline changes were observed.

W. R. S.

## CURRENT BACTERIOLOGICAL LITERATURE.

H. W. CONN, Wesleyan University.

Separates of Papers and Books on Bacteriology should be Sent for Review to H. W. Conn,  
Wesleyan University, Middletown, Conn.

**Gabritschewsky.** Beiträge zu bakteriologischen untersuchungsmethoden. Cent. f. Bac. u. Par. I, 31: 6, 813.

The use of heat for fixing bacteria upon cover-glasses previous to staining is known to have an influence upon the

power of bacteria in absorbing stains; too high a heat rendering the bacteria incapable of such absorption. The author has tested this property by placing covers of various organisms in a culture oven where the temperature can be kept at a known point, and then testing the staining power of the organisms by subsequent treatment. He experiments with a variety of bacteria and finds, with some variations, that bacteria color well if heated to a temperature not over 170–180°, that spores will color when heated to a temperature still higher than this, but that at a temperature of 220°, neither spores nor bacilli will stain. The power of staining by the Gram method disappears at a higher temperature than that of ordinary staining. In general, if heated to a temperature of over 220°, the power of absorbing stains is completely lost.

H. W. C.

**Onellanski.** Die Kultur der Nitritbildners auf Papierscheiben. Cent. f. Bac. u. Par. II, 8: 786.

In experimentation with the so-called nitro bacteria it has been necessary to develop special culture media, inas-

much as these organisms will not grow in the presence of organic material and are therefore quite unadapted to the common media of the laboratory. Winogradski has used culture media of gelatinized silica. The author of this paper has developed a new method of cultivating these organisms by the use of common filter paper. He takes a thick pack of common filters, sews them together at their edge, and places them in a petri dish. In the bottom of the petri dish he places carbonate of magnesia and then pours into the dish, so as to partly cover the filter papers, the nitrogen free culture media designed for the development of the bacteria. He inoculates the surface of the filter papers with the material to be tested and finds that the organisms grow readily and soon make their appearance as isolated colonies on the surface of the filter paper which can subsequently be separated and experimented with. He finds it also possible to use strips of filter paper in test tube cultures and regards the use of such paper as a decided advantage in the experimenting with the nitro bacteria. The method is useful in cultivating the nitrous bacteria, but not those that produce nitric acid.

H. W. C.

**MacFarland.** Tetanus and Vaccination. Jour. Med. Research, 2: 474, 1902.

The widespread interest excited in the last year by the occurrence of tetanus

following vaccination gives special importance to this careful analysis of the cases, and conclusions as to the cause. The author concludes, after very careful study, that the recent cases of tetanus are to be attributed to the vaccine virus

and not to secondary contamination of the vaccine sores. He finds that the larger part of them came from the virus from one maker, and concludes that it is probably due to the rare case of the contamination of the calves from which the virus was taken with the tetanus bacillus. A final foot-note states that Dr. R. W. Wilson has succeeded in actually finding the tetanus bacillus in some of the same vaccine used at the time of the chief outbreak in 1901. H. W. C.

**Von Wendt.** Ueber eine einfache Methode, Bakterien ohne Trocknen an Deck- oder Objectgläser zu fixieren. *Cent. f. Bac.* I, 31: 0, 671, 1902.

The heating bacteria in the ordinary fixing method undoubtedly produces slight modifications in the organism so that the subsequent microscopic appearance is somewhat modified.

The author has devised a method of fixing the bacteria without the use of heat in order to facilitate the careful microscopic study. The method is briefly as follows: A loopful of bacteria culture is placed in a drop of water or in water containing 3 per cent. nitric acid and one-half per cent. corrosive sublimate. A cover-glass is then covered with a thin layer of Meyer's albumin glycerine fixative, a drop of water is placed upon the cover glass and a small amount of the bacteria mixture is placed carefully upon the drop of water with the platinum loop. The bacteria spread through the drop and gradually sink. The whole is placed under a watch glass for half an hour when the bacteria will have sunk to the bottom of the drop. It is then placed, still under the watch glass, in an oven at the temperature of 75° for 8 to 10 minutes to coagulate the albumen. This must be done under conditions which prevent the water from evaporating in order to keep the bacteria moist. The water may then be removed and staining reagent applied, for the bacteria will be fixed by the albumen. H. W. C.

**Drigalski and Conradl.** Ueber ein beifahren zum Nachweis der Typhusbacillen. *Zeit. f. Hyg. u. Infec.* 29: 283, 1902.

The authors have devised a method of distinguishing typhoid bacillus based upon somewhat different principle from any of the other methods used.

It is dependent upon the use of a culture medium which especially stimulates the growth of the typhoid bacillus, the essential features of which are the use of a larger per cent. of agar and bouillon than usual, the presence of an alkaline albuminate (nutrose) and of crystals violet B. The medium is made with 3 pounds of chopped beef, 2 liters of water, 20 grams of peptone, 10 grams of salt, 20 grams of nutrose, and 60 of agar. A second solution contains 260 c. c. of litmus solution and 30 grams of milk sugar. These two solutions are mixed and rendered alkaline, after which 20 c. c. of a one-tenth solution of crystal violet B is added. The typhoid colonies develop readily on such plates and are easily differentiated by the agglutination test after 24 hours' growth. With this material he is able to separate the typhoid bacillus in a large number of cases from the body excretions before there are distinct symptoms in the patient and in many cases where the Widal test has failed. H. W. C.

**Castellani.** Upon a Special Method for the Detection of the Typhoid Bacillus in the Blood. *Cent. f. Bac. u. Par.* I, 31: 0, 477.

This method depends upon a dilution of the blood sufficiently to remove the disturbing factor of the agglutinating bodies.

A few centimeters of blood of the patient is added to several large flasks each containing 30 c. c. of faintly alkaline beef broth. The flasks are incubated at blood heat, and in 12 cases out of 14 the typhoid bacillus was detected by subsequent study. H. W. C.

## NEWS AND NOTES.

**PROFESSIONAL SPIRIT AND PUBLICATION.**—The September monthly letter to collaborators sent out by this journal contains a statement that is worthy of more than passing notice or mere financial interest. Here it stated that a magazine formed for the express purpose of being mutually helpful to science teachers and investigators, and continuing true to the purpose for which it was founded will, in all probability, have to double its price of subscription in order to continue publication. Wherein lies the difficulty? Certainly not in the periodical itself. A perusal of the table of contents since the appearance of the first number makes such a thought impossible. The list of contributors is of itself a guarantee of sterling worth, while the titles of the articles published show a remarkably wide range of subjects covered in a way that could not but be of real help and inspiration to any science teacher or worker who is desirous of keeping abreast of the newest and best in matter and method.

The fault, then, must surely lie with the very persons whom it was hoped to reach and benefit by this journal. David Harum said: "You 'ain't out of danger of gettin' in a rut," and this bit of wisdom seems to be applicable to teachers in secondary schools with no little force. Progress demands contact with current men and events. Suggestion from others is essential to advancement. Experience is of greatest value only when it is supplemented and enlarged by the experience of others. A recognition of these truths leads only to one conclusion, and that is that contact with other workers is absolutely essential if the science teacher is to make any progress. It is not enough to know what has been found out, how things used to be done, one way of doing things; but what, in addition, is now being discovered, how are results accomplished to-day in better ways than formerly, in how many different ways may a certain result be obtained. Resourcefulness is one of the greatest needs of science teachers as of all other teachers at present. And so it is essential for the teacher and worker to read, not books only, but current literature as found in magazines.

It would seem as if any one whose chosen life work was in science or the teaching of science would not need to have this said, but would have felt the force of it all sufficiently to act accordingly. But, "there's the rub," and it is not a pleasant thing for our teachers to contemplate. It may not, perhaps, be an exaggeration to say that the majority of science teachers, especially in secondary schools, are not engaged in their life vocation, but in an avocation which for some may serve as a stepping stone to a principalship or to law or medicine, or, it may be, as an easy source of income until marriage shall bring relief from the confinement and care of the class-room and laboratory. As long as school organization permits these conditions to continue so long will the development of the professional spirit be delayed, and with professional spirit existing only in books on Pedagogy it is quite too much to expect that those engaged in teaching (they can hardly be called teachers) will attend conventions and support professional publications, or contribute in any other way to the advancement and dignity of the calling they so lamely follow.

It seems a shame that a journal like this should have to increase its price of subscription merely to pay expenses. The field is not overcrowded. Publications are yet too few to supply what ought to be the demand, and this journal ought to receive the support of every progressive teacher and laboratory. The writer is not a pessimist, for he does not believe that the existing conditions are permanent or that they are as bad as they have been, but one gets impatient sometimes for the better things of which one catches a glimpse.

## QUESTION BOX.

Inquiries will be printed in this department from any inquirer.  
The replies will appear as received.

No. 27. What is Knop's solution?

### REPLY TO QUESTION No. 25.

In the Question Box of the JOURNAL OF APPLIED MICROSCOPY for June, I noticed an inquiry for a satisfactory method of mounting simple microscopical objects such as fern and mushroom spores, etc. Thinking that the methods I have found satisfactory for mounting fern spores and pollen of flowers might be helpful to the inquirer, I will briefly describe them. I may say, first, that I always keep a quantity of slides on hand all ready for use. These slides are prepared by first cleaning them, then placing on a turn-table and laying on a ring of gold size, thus forming a very shallow cell of the proper size for the cover-glasses which are to be used. To mount spores or pollen, I take one of these prepared slides and taking up a small quantity of glycerine jelly on the point of a knife place it on the slide in the center of the gold size cell. The slide is then placed on the warming table and allowed to remain until the jelly melts, when it is removed, allowed to cool slightly, and a small quantity of the spores or pollen stirred into the melted jelly by the aid of a needle. A clean cover-glass is then taken up with a pair of forceps, held over the lamp for a few moments to warm it, and placed on the slide, over the object, by bringing one edge of the cover into contact with the ring of gold size at one side and allowing it slowly and gently to fall into place. A spring clip is then placed on the slide to hold the cover-glass securely in place, and the whole put aside under a bell-glass until the jelly has hardened. I usually, if not in a hurry, allow them to remain a day or two—when any superfluous jelly may be removed with a sharp-pointed knife and the cover sealed down by running a ring of gold size around its edge. This, when the gold size has dried, completes the slide with the exception of labeling, and, if desired, finishing with ring of colored varnish. The other method I use for the same class of objects is similar to the above except that Canada balsam, prepared as follows, is used instead of glycerine jelly.

Canada Balsam,	-	-	-	-	-	1½ ounce.
Turpentine,	-	-	-	-	-	½ "
Chloroform,	-	-	-	-	-	½ "

The Canada balsam prepared in this way, being liquid, requires no melting; simply place a drop on the slide, stir in the spores or pollen, and then proceed as with the glycerine jelly. The only difficulty I have found with the glycerine jelly is that considerable care is required to avoid air bubbles. With the Canada balsam this difficulty is almost entirely removed, but a longer time is required for the slides to dry so that the covers may be sealed down.

These methods, especially the latter, I have found very satisfactory, not only for the objects mentioned, but for many others, such as sections of animal and vegetable substances, parts of insects, etc.

L. E. COFFIN.

Center Tuftonboro, N. H.





3 gal  
174